Customer No. 20350
TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
(415) 576-0200

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Transmitted herewith for filing under 37 CFR 1.53(b) is the
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[X] continuation-in-part patent application of

Inventor(s)/Applicant Identifier: Alessandro SETTE, John SIDNEY, Scott SOUTHWOOD, Maria A. VITIELLO, Brian D. LIVINGSTON, Esteban CELIS, Ralph T. KUBO, Howard M. GREY, and Robert CHESNUT

For: INDUCING CELLULAR IMMUNE RESPONSES TO HEPATITIS B VIRUS USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS

[X] This application claims priority from each of the following Application Nos./filing dates: 08/820,360, filed March 12,1997; 60/013,363, filed March 13, 1996; 09/189,702, filed November 10, 1998; 08/205,713, filed March 4, 1994; 08/159,184, filed November 29, 1993; 08/073,205, filed June 4, 1993; and 08/027,146, filed March 5, 1993.,the disclosure(s) of which is (are) incorporated by reference.

[ ] Please amend this application by adding the following before the first sentence: "This application is a [ ] continuation [ ] continuation-in-part of and claims the benefit of U.S. Application No. 60/_____, filed _____, the disclosure of which is incorporated by reference."

Enclosed are:

[X] 208 page(s) of specification
[X] 6 page(s) of claims
[X] 1 page of Abstract
[X] 2 sheet(s) of [ ] formal [X] informal drawing(s).
[ ] An assignment of the invention to _____
[ ] A [ ] signed [ ] unsigned Declaration & Power of Attorney
[X] A [ ] signed [X] unsigned Declaration.
[ ] A Power of Attorney.
[ ] A verified statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27 [ ] is enclosed [ ] was filed in the prior application and small entity status is still proper and desired.
[ ] A certified copy of a _____application.
[ ] Information Disclosure Statement under 37 CFR 1.97.
[ ] A petition to extend time to respond in the parent application.
[ ] Notification of change of [ ] power of attorney [ ] correspondence address filed in prior application.
[ ]

In view of the Unsigned Declaration as filed with this application and pursuant to 37 CFR §1.53(f), Applicant requests deferral of the filing fee until submission of the Missing Parts of Application.

DO NOT CHARGE THE FILING FEE AT THIS TIME.

Jean M. Lockyer, Reg No.: P-44,879
Attorneys for Applicant

Telephone:
(415) 576-0200

Facsimile:
(415) 576-0300

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PATENT APPLICATION

INDUCING CELLULAR IMMUNE RESPONSES TO HEPATITIS B VIRUS USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS

Inventor(s): Alessandro Sette, an Italian citizen, residing at
5551 Linda Rosa Avenue
La Jolla, California 92037

John Sidney, a United States citizen, residing at
4218 Corte de la Siena
La Jolla, California 92130

Scott Southwood, a United States citizen, residing at
10679 Strathmore Drive
Santee, California 92071

Maria A. Vitiello, an Italian citizen, residing at
7522 High Avenue
La Jolla, CA 92037

Brian D. Livingston, a United States citizen, residing at
13555 Chaco Court
San Diego, California 92129

Esteban Celis, a United States citizen, residing at
3683 Wright Road S.W.
Rochester, Minnesota 55902

Ralph T. Kubo, a United States citizen, residing at
6921 Pear Tree Drive
Carlsbad, California 92009

Howard M. Grey, a United States citizen, residing at
1461 Caminito Batea
La Jolla, California 92037

Robert Chesnut, a United States citizen, residing at
1473 Kings Cross Drive
Cardiff-by-the-Sea, California 92007

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
(415) 576-0200

PATENT

Attorney Docket No.: 018623-013900US

INDUCING CELLULAR IMMUNE RESPONSES TO HEPATITIS B VIRUS USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a Continuation-In-Part ("CIP") of U.S.S.N. 08/820,360 filed 3/12/97, which claims the benefit of U.S. Provisional Application No. 60/013,363 filed March 13, 1996 and now abandoned. The present application is also a CIP of U.S.S.N. 09/189,702 filed 11/10/98, which is a CIP of U.S.S.N 08/205,713 filed 3/4/94, which is a CIP of 08/159,184 filed 11/29/93 and now abandoned, which is a CIP of 08/073,205 filed 6/4/93 and now abandoned, which is a CIP of 08/027,146 filed 3/5/93 and now abandoned. The present application is also related to U.S.S.N. 08/197,484, U.S.S.N. 08/464,234, U.S.S.N. 08/464,496, U.S.S.N. 08/464,031, abandoned U.S.S.N. 08/464,433, and U.S.S.N. 08/461,603, which is a continuation of abandoned U.S.S.N. 07/935,811, which is a CIP of abandoned U.S.S.N. 07/874,491, which is a CIP of abandoned U.S.S.N. 07/827,682, which is a CIP of abandoned 07/749,568. The present application is also related to U.S. Patent Application entitled "Peptides and Methods for Creating Synthetic Peptides with Modulated Binding Affinity for HLA Molecules", Attorney Docket No. 018623-009520, filed 1/6/99, which is a CIP of U.S.S.N. 08/815,396, which is a CIP of abandoned U.S.S.N. 60/013,113. Furthermore, the present application is related to U.S.S.N. 09/017,735, which is a CIP of abandoned U.S.S.N. 08/589,108; U.S.S.N. 08/753,622, U.S.S.N. 08/822,382, abandoned U.S.S.N. 60/013,980, U.S.S.N. 08/454,033, U.S.S.N. 09/116,424, U.S.S.N. 08/205,713, and U.S.S.N. 08/349,177, which is a CIP of abandoned U.S.S.N. 08/159,184, which is a CIP of abandoned U.S.S.N. 08/073,205, which is a CIP of abandoned U.S.S.N. 08/027,146. The present application is also related to U.S.S.N. 09/017,524, U.S.S.N. 08/821,739, abandoned U.S.S.N. 60/013,833, U.S.S.N. 08/758,409, U.S.S.N. 08/589,107, U.S.S.N. 08/451,913, U.S.S.N. 08/186,266, U.S.S.N. 09/116,061, and U.S.S.N. 08/347,610, which is a CIP of U.S.S.N. 08/159,339, which is a CIP of abandoned U.S.S.N. 08/103,396, which is a CIP of abandoned U.S.S.N. 08/027,746, which is a CIP of abandoned U.S.S.N. 07/926,666. The present application is also related to U.S.S.N. 09/017,743, U.S.S.N. 08/753,615; U.S.S.N. 08/590,298, U.S.S.N. 09/115,400, and U.S.S.N. 08/452,843, which is a CIP of U.S.S.N.

08/344,824, which is a CIP of abandoned U.S.S.N. 08/278,634. The present application is also related to provisional U.S.S.N. 60/087,192 and U.S.S.N. 09/009,953, which is a CIP of abandoned U.S.S.N. 60/036,713 and abandoned U.S.S.N. 60/037,432. In addition, the present application is related to U.S.S.N. 09/098,584 and to Provisional U.S.S.N. 60/117,486. All of the above applications are incorporated herein by reference.

FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was funded, in part, by the United States government under grants with the National Institutes of Health. The U.S. government has certain rights in this invention.

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I. BACKGROUND OF THE INVENTION

Chronic infection by hepatitis B virus (HBV) affects at least 5% of the world's population and is a major cause of cirrhosis and hepatocellular carcinoma (Hoofnagle, J., *N. Engl. J. Med.* 323:337, 1990; Fields, B. and Knipe, D., In: *Fields Virology* 2:2137, 1990). The World Health Organization lists hepatitis B as a leading cause of death worldwide, close behind chronic pulmonary disease, and more prevalent than AIDS. Chronic HBV infection can range from an asymptomatic carrier state to continuous hepatocellular necrosis and inflammation, and can lead to hepatocellular carcinoma.

The immune response to HBV is believed to play an important role in controlling hepatitis B infection. A variety of humoral and cellular responses to different regions of HBV including the nucleocapsid core, polymerase, and surface antigens have been identified. T cell-mediated immunity, particularly involving class I human leukocyte antigen-restricted cytotoxic T lymphocytes (CTL), is believed to be crucial in combatting established HBV infection.

Class I human leukocyte antigen (HLA) molecules are expressed on the surface of almost all nucleated cells. CTL recognize peptide fragments, derived from intracellular processing of various antigens, in the form of a complex with class I HLA molecules. This recognition event then results in the destruction of the cell bearing the HLA-peptide complex directly or the activation of non-destructive mechanisms e.g., the production of interferon, that inhibit viral replication.

Several studies have emphasized the association between self-limiting acute hepatitis and multispecific CTL responses (Penna, A. *et al.*, *J. Exp. Med.* 174:1565, 1991; Nayersina, R. *et al.*, *J. Immunol.* 150:4659, 1993). Spontaneous and interferon-related clearance of chronic HBV infection is also associated with the resurgence of a vigorous CTL response (Guidotti, L. G. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:3764, 1994). In all such cases the CTL responses are polyclonal, and specific for multiple viral proteins including the HBV envelope, core and polymerase antigens. By contrast, in patients with chronic hepatitis, the CTL activity is usually absent or weak, and antigenically restricted.

The crucial role of CTL in resolution of HBV infection has been further underscored by studies using HBV transgenic mice. Adoptive transfer of HBV-specific CTL into mice transgenic for the HBV genome resulted in suppression of virus replication. This effect was primarily mediated by a non-lytic, lymphokine-based mechanism (Guidotti, L. G. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:3764, 1994; Guidotti, L.

G., Guilhot, S., and Chisari, F. V. *J. Virol.* 68:1265, 1994; Guidotti, L. G. *et al.*, *J. Virol.* 69:6158, 1995; Gilles, P. N., Fey, G., and Chisari, F. V., *J. Virol.* 66:3955, 1992).

As is the case for HLA class I restricted responses, HLA class II restricted T cell responses are usually detected in patients with acute hepatitis, and are absent or weak in patients with chronic infection (Chisari, F. V. and Ferrari, C., *Annu. Rev. Immunol.* 13:29, 1995). HLA Class II responses are tied to activation of helper T cells (HTLs) Helper T lymphocytes, which recognize Class II HLA molecules, may directly contribute to the clearance of HBV infection through the secretion of cytokines which suppress viral replication (Franco, A. *et al.*, *J. Immunol.* 159:2001, 1997). However, their primary role in disease resolution is believed to be mediated by inducing activation and expansion of virus-specific CTL and B cells.

In view of the heterogeneous immune response observed with HBV infection, induction of a multi-specific cellular immune response directed simultaneously against multiple epitopes appears to be important for the development of an efficacious vaccine against HBV. There is a need to establish vaccine embodiments that elicit immune responses that correspond to responses seen in patients that clear HBV infection. Epitope-based vaccines appear useful.

Upon development of appropriate technology, the use of epitope-based vaccines has several advantages over current vaccines. The epitopes for inclusion in such a vaccine are to be selected from conserved regions of viral or tumor-associated antigens, in order to reduce the likelihood of escape mutants. The advantage of an epitope-based approach over the use of whole antigens is that there is evidence that the immune response to whole antigens is directed largely toward variable regions of the antigen, allowing for immune escape due to mutations. Furthermore, immunosuppressive epitopes that may be present in whole antigens can be avoided with the use of epitope-based vaccines.

Additionally, with an epitope-based vaccine approach, there is an ability to combine selected epitopes (CTL and HTL) and additionally to modify the composition of the epitopes, achieving, for example, enhanced immunogenicity. Accordingly, the immune response can be modulated, as appropriate, for the target disease. Similar engineering of the response is not possible with traditional approaches.

Another major benefit of epitope-based immune-stimulating vaccines is their safety. The possible pathological side effects caused by infectious agents or whole protein antigens, which might have their own intrinsic biological activity, is eliminated.

An epitope-based vaccine also provides the ability to direct and focus an immune response to multiple selected antigens from the same pathogen. Thus, patient-by-patient variability in the immune response to a particular pathogen may be alleviated by inclusion of epitopes from multiple antigens from that pathogen in a vaccine composition. A "pathogen" may be an infectious agent or a tumor associated molecule.

However, one of the most formidable obstacles to the development of broadly efficacious epitope-based immunotherapeutics has been the extreme polymorphism of HLA molecules. To date, effective non-genetically biased coverage of a population has been a task of considerable complexity; such coverage has required that epitopes be used specific for HLA molecules corresponding to each individual HLA allele, therefore, impractically large numbers of epitopes would have to be used in order to cover ethnically diverse populations. There has existed a need to develop peptide epitopes that are bound by multiple HLA antigen molecules for use in epitope-based vaccines. The greater the number of HLA antigen molecules bound, the greater the breadth of population coverage by the vaccine.

Furthermore, as described herein in greater detail, a need has existed to modulate peptide binding properties, for example so that peptides that are able to bind to multiple HLA antigens do so with an affinity that will stimulate an immune response. Identification of epitopes restricted by more than one HLA allele at an affinity that correlates with immunogenicity is important to provide thorough population coverage, and to allow the elicitation of responses of sufficient vigor whereby the natural immune responses noted in self-limiting acute hepatitis, or of spontaneous clearance of chronic HBV infection is induced in a diverse segment of the population. Such a response can also target a broad array of epitopes. The technology disclosed herein provides for such favored immune responses.

The information provided in this section is intended to disclose the presently understood state of the art as of the filing date of the present application. Information is included in this section which was generated subsequent to the priority date of this application. Accordingly, background in this section is not intended, in any way, to delineate the priority date for the invention.

II. SUMMARY OF THE INVENTION

This invention applies our knowledge of the mechanisms by which antigen is recognized by T cells, for example, to develop epitope-based vaccines directed towards

HBV. More specifically, this application communicates our discovery of specific epitope pharmaceutical compositions and methods of use in the prevention and treatment of HBV infection.

Upon development of appropriate technology, the use of epitope-based vaccines has several advantages over current vaccines, particularly when compared to the use of whole antigens in vaccine compositions. There is evidence that the immune response to whole antigens is directed largely toward variable regions of the antigen, allowing for immune escape due to mutations. The epitopes for inclusion in an epitope-based vaccine are selected from conserved regions of viral or tumor-associated antigens, which thereby reduces the likelihood of escape mutants. Furthermore, immunosuppressive epitopes that may be present in whole antigens can be avoided with the use of epitope-based vaccines.

An additional advantage of an epitope-based vaccine approach is the ability to combine selected epitopes (CTL and HTL), and further, to modify the composition of the epitopes, achieving, for example, enhanced immunogenicity. Accordingly, the immune response can be modulated, as appropriate, for the target disease. Similar engineering of the response is not possible with traditional approaches.

Another major benefit of epitope-based immune-stimulating vaccines is their safety. The possible pathological side effects caused by infectious agents or whole protein antigens, which might have their own intrinsic biological activity, is eliminated.

An epitope-based vaccine also provides the ability to direct and focus an immune response to multiple selected antigens from the same pathogen. Thus, patient-by-patient variability in the immune response to a particular pathogen may be alleviated by inclusion of epitopes from multiple antigens from that pathogen in a vaccine composition. A "pathogen" may be an infectious agent or a tumor associated molecule.

One of the most formidable obstacles to the development of broadly efficacious epitope-based immunotherapeutics, however, has been the extreme polymorphism of HLA molecules. To date, effective non-genetically biased coverage of a population has been a task of considerable complexity; such coverage has required that epitopes be used specific for HLA molecules corresponding to each individual HLA allele, therefore, impractically large numbers of epitopes would have to be used in order to cover ethnically diverse populations. Thus, there has existed a need to develop peptide epitopes that are bound by multiple HLA antigen molecules for use in epitope-based vaccines. The greater the number of HLA antigen molecules bound, the greater the breadth of population coverage by the vaccine.

Furthermore, as described herein in greater detail, a need has existed to modulate peptide binding properties, for example, so that peptides that are able to bind to multiple HLA antigens do so with an affinity that will stimulate an immune response. Identification of epitopes restricted by more than one HLA allele at an affinity that correlates with immunogenicity is important to provide thorough population coverage, and to allow the elicitation of responses of sufficient vigor to prevent or clear an infection in a diverse segment of the population. Such a response can also target a broad array of epitopes. The technology disclosed herein provides for such favored immune responses.

In a preferred embodiment, epitopes for inclusion in vaccine compositions of the invention are selected by a process whereby protein sequences of known antigens are evaluated for the presence of motif or supermotif-bearing epitopes. Peptides corresponding to a motif- or supermotif-bearing epitope are then synthesized and tested for the ability to bind to the HLA molecule that recognizes the selected motif. Those peptides that bind at an intermediate or high affinity *i.e.*, an IC_{50} (or a K_D value) of 500 nM or less for HLA class I molecules or 1000 nM or less for HLA class II molecules, are further evaluated for their ability to induce a CTL or HTL response. Immunogenic peptides are selected for inclusion in vaccine compositions.

Supermotif-bearing peptides may additionally be tested for the ability to bind to multiple alleles within the HLA supertype family. Moreover, peptide epitopes may be analogued to modify binding affinity and/or the ability to bind to multiple alleles within an HLA supertype.

The invention also includes an embodiment comprising a method for monitoring immunogenic activity of a vaccine for HBV in a patient having a known HLA-type, the method comprising incubating a T lymphocyte sample from the patient with a peptide composition comprising an HBV epitope consisting essentially of an amino acid sequence described in Tables VI to Table XX or Table XXII which binds the product of at least one HLA allele present in said patient, and detecting for the presence of a T lymphocyte that binds to the peptide. In a preferred embodiment, the peptide comprises a tetrameric complex.

An alternative modality for defining the peptides in accordance with the invention is to recite the physical properties, such as length; primary, potentially secondary and/or tertiary structure; or charge, which are correlated with binding to a particular allele-specific HLA molecule or group of allele-specific HLA molecules. A further modality for defining peptides is to recite the physical properties of an HLA binding pocket, or

properties shared by several allele-specific HLA binding pockets (*e.g.* pocket configuration and charge distribution) and reciting that the peptide fits and binds to said pocket or pockets.

As will be apparent from the discussion below, other methods and embodiments are also contemplated. Further, novel synthetic peptides produced by any of the methods described herein are also part of the invention.

III. BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Figure 1 provides a graph of total frequency of genotypes as a function of the number of HBV candidate epitopes bound by HLA-A and B molecules, in an average population.

Figure 2: Figure 2 Illustrates the Position of Peptide Epitopes in Experimental Model Minigene Constructs

IV. DETAILED DESCRIPTION OF THE INVENTION

The peptides and corresponding nucleic acid compositions of the present invention are useful for stimulating an immune response to HBV either by stimulating the production of CTL or HTL responses. The peptides, which are derived directly or indirectly from native HBV amino acid sequences, are able to bind to HLA molecules and stimulate an immune response to HBV. The complete polyprotein sequence from HBV and its variants can be obtained from Genbank. Peptides can also be readily determined from sequence information that may subsequently be discovered for heretofore unknown variants of HBV as will be clear from the disclosure provided below.

The peptides of the invention have been identified in a number of ways, as will be discussed below. Further, analog peptides have been derived and the binding activity for HLA molecules modulated by modifying specific amino acid residues to create peptide analogs exhibiting altered immunogenicity. Further, the present invention provides compositions and combinations of compositions that enable epitope-based vaccines that are capable of interacting with multiple HLA antigens to provide broader population coverage than prior vaccines.

IV.A. Definitions

The invention can be better understood with reference to the following definitions, which are listed alphabetically.

"Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

A "cryptic epitope" elicits a response by immunization with an isolated peptide, but the response is not cross-reactive *in vitro* when intact whole protein which comprises the epitope is used as an antigen.

A "dominant epitope" is an epitope that induces an immune response upon immunization with a whole native antigen.. (See, *e.g.*, Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729766 (1993)) Such a response is cross-reactive *in vitro* with an isolated peptide epitope.

With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. In an immune system setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site recognized by an immunoglobulin, T cell receptor or HLA molecule.

"Human Leukocyte Antigen" or "HLA" is a human class I or class II Major Histocompatibility Complex (MHC) protein (*see*, Stites, *et al.*, IMMUNOLOGY, 8TH ED., Lange Publishing, Los Altos, CA (1994).

An "HLA supertype or family", as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into HLA supertypes. The terms HLA superfamily, HLA supertype family, and HLA xx-like supertype molecules (where xx denotes a particular HLA type) are synonyms.

Throughout this disclosure, results are expressed in terms of "IC_{50}'s." IC_{50} is the concentration of peptide in a binding assay at which 50% inhibition of binding of a reference peptide is observed. Given the conditions in which the assays are run (*i.e.*, limiting HLA proteins and labeled peptide concentrations), these values approximate K_D values. It should be noted that IC_{50} values can change, often dramatically, if the assay conditions are varied, and depending on the particular reagents used (*e.g.*, HLA preparation, *etc.*). For example, excessive concentrations of HLA molecules will increase the apparent measured IC_{50} of a given ligand.

Assays for determining binding are described in detail in PCT publications WO 94/20127 and WO 94/03205. Alternatively, binding is expressed relative to a reference peptide. As a particular assay becomes more, or less, sensitive, the IC_{50}'s of the peptides tested may change somewhat. However, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC_{50} of the reference peptide increases 10-fold, the IC_{50} values of the test peptides will also shift approximately 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC_{50}, relative to the IC_{50} of a standard peptide.

Binding may also be determined using other assays including, for example, inhibition of antigen presentation (Sette *et al.*, *J. Immunol.* 141:3893, 1991), *in vitro* assembly assays (Townsend *et al.*, *Cell* 62:285, 1990), measures of dissociations rates (Parker *et al.*, *J. Immunol.* 149:1896-1904, 1992), and FACS-based assays using mutated cells, such as RMA.S (Melief, *et al.*, *Eur. J. Immunol.* 21:2963, 1991).

As used herein, high affinity with respect to HLA class I molecules is defined as binding with an IC_{50} or K_D value of less than 50 nM; intermediate affinity is binding with an IC_{50} (or K_D) of between about 50 and about 500 nM. High affinity with respect to binding to HLA class II molecules is defined as binding with an IC_{50} or K_D value of less than 100 nM; intermediate affinity is binding with an IC_{50} or K_D of between about 100 and about 1000 nM.

The terms "identical" or percent "identity," in the context of two or more peptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithms or by manual alignment and visual inspection.

An "immunogenic peptide" or "peptide epitope" is a peptide which comprises an allele-specific motif or supermotif such that the peptide will bind an HLA molecule and induce a CTL and/or HTL response. Thus, immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and thereafter inducing a cytotoxic T cell response, or a helper T cell response, to the antigen from which the immunogenic peptide is derived.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention

preferably do not contain materials normally associated with the peptides in their *in situ* environment.

"Major Histocompatibility Complex" or "MHC" is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the MHC complex is also known as the HLA complex. For a detailed description of the MHC and HLA complexes, see, Paul, FUNDAMENTAL IMMUNOLOGY, 3RD ED., Raven Press, New York, 1993.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

A "negative binding residue" or "deleterious residue" is an amino acid which, if present at certain positions (typically not primary anchor positions) of a peptide epitope, results in decreased binding affinity of the peptide for the peptide's corresponding HLA molecule. Any residue that is not "deleterious" is a "non-deleterious" residue.

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α-amino and carboxyl groups of adjacent amino acids. The preferred CTL-inducing oligopeptides of the invention are 13 residues or less in length and usually consist of between about 8 and about 11 residues, preferably 9 or 10 residues. The preferred HTL-inducing oligopeptides are less than about 50 residues in length and usually consist of between about 6 and about 30 residues, more usually between about 12 and 25, and often between about 15 and 20 residues.

"Pharmaceutically acceptable" refers to a non-toxic, inert, and physiologically compatible composition.

A "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic peptide and the HLA molecule. One to three, usually two, primary anchor residues within a peptide of defined length generally defines a "motif" for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves themselves. In one embodiment, the primary anchor residues are located

at position 2 (from the amino terminal position) and at the carboxyl terminal position of a 9 residue peptide in accordance with the invention. The primary anchor positions for each motif and supermotif are set forth in Table I. For example, analog peptides can be created by altering the presence or absence of particular residues in these primary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a particular motif or supermotif.

"Promiscuous recognition" is where a distinct peptide is recognized by the same T cell clone in the context of multiple HLA molecules. Promiscuous binding is synonymous with cross-reactive binding.

A "protective immune response" or "therapeutic immune response" refers to a CTL and/or an HTL response to an antigen from an infectious agent or a tumor antigen from which an immunogenic peptide is derived, and thereby preventing or at least partially arresting disease symptoms or progression. The immune response may also include an antibody response which has been facilitated by the stimulation of helper T cells.

The term "residue" refers to an amino acid or amino acid mimetic incorporated into an oligopeptide by an amide bond or amide bond mimetic.

A "secondary anchor residue" is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor residue occurs at a significantly higher frequency amongst bound peptides than would be expected by random distribution of amino acids at one position. The secondary anchor residues are said to occur at "secondary anchor positions." A secondary anchor residue can be identified as a residue which is present at a higher frequency among high affinity binding peptides, or a residue otherwise associated with high affinity binding. For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a particular motif or supermotif.

A "subdominant epitope" is an epitope which evokes little or no response upon immunization with whole antigens which comprise the epitope, but for which a response can be obtained by immunization with an isolated peptide, and this response (unlike the case of cryptic epitopes) is detected when whole protein is used to recall the response *in vitro* or *in vivo*.

A "supermotif" is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles. A supermotif-bearing epitope is preferably is recognized with high or intermediate affinity (as defined herein) by two or more HLA antigens.

"Synthetic peptide" refers to a peptide that is not naturally occurring, but is man-made using such methods as chemical synthesis or recombinant DNA technology.

The nomenclature used to describe peptide compounds follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxyl group to the right (the C-terminus) of each amino acid residue. When amino acid residue positions are referred to in a peptide epitope they are numbered in an amino to carboxyl direction with position one being the position closest to the amino terminal. In the formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G. Symbols for the amino acids are shown below.

Single Letter Symbol	Three Letter Symbol	Amino Acids
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic Acid
E	Glu	Glutamic Acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

IV.B. Stimulation of CTL and HTL responses against HBV

The mechanism by which T cells recognize antigens has been delineated during the past ten years. Based on our new understanding of the immune system we have generated efficacious peptide epitope vaccine compositions that can induce a therapeutic or prophylactic immune response to HBV infection in a broad population. For an understanding of the value and efficacy of the claimed compositions, a brief review of the technology is provided.

A complex of an HLA molecule and a peptidic antigen acts as the ligand recognized by HLA-restricted T cells (Buus, S. *et al.*, *Cell* 47:1071, 1986; Babbitt, B. P. *et al.*, *Nature* 317:359, 1985; Townsend, A., and Bodmer, H., *Annu. Rev. Immunol.* 7:601,

1989; Germain, R. N., *Annu. Rev. Immunol.* 11:403, 1993). Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues that correspond to motifs required for specific binding to HLA antigen molecules have been identified and are described here and set forth in Tables I, II, and III (see also, e.g., Sette, A. and Grey, H. M., *Curr. Opin. Immunol.* 4:79, 1992; Sinigaglia, F. and Hammer, J., *Curr. Biol.* 6:52, 1994; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994). Furthermore, x-ray crystallographic analysis of HLA-peptide complexes has revealed pockets within the peptide binding cleft of HLA molecules which accommodate allele-specific residues borne by peptide ligands; these residues in turn determine the HLA binding capacity of the peptides in which they are present (Brown, J. H. *et al.*, *Nature* 364:33, 1993; Guo, H. C. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:8053, 1993; Guo, H. C. *et al.*, *Nature* 360:364, 1992; Silver, M. L. *et al.*, *Nature* 360:367, 1992; Matsumura, M. *et al.*, *Science* 257:927, 1992; Madden *et al.*, *Cell* 70:1035, 1992; Fremont, D. H. *et al.*, *Science* 257:919, 1992; Saper, M. A. , Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991).

Accordingly, the definition of class I and class II allele-specific HLA binding motifs or class I supermotifs allows identification of regions within a protein that have the potential of binding particular HLA antigens (see also *e.g.*, Sette, A. and Grey, H. M., *Curr. Opin. Immunol.* 4:79, 1992; Sinigaglia, F. and Hammer, J., *Curr. Biol.* 6:52, 1994; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994; Kast, W. M. *et al.*, *J. Immunol.*, 152:3904, 1994).

Furthermore, a variety of assays to quantify the affinity of interaction between peptide and HLA have also been established. Such assays include, for example, measures of IC_{50} values, inhibition of antigen presentation (Sette *et al.*, *J. Immunol.* 141:3893, 1991), *in vitro* assembly assays (Townsend *et al.*, *Cell* 62:285, 1990), measures of dissociations rates (Parker *et al.*, *J. Immunol.* 149:1896-1904, 1992), and FACS-based assays using mutated cells, such as RMA.S (Melief, *et al.*, *Eur. J. Immunol.* 21:2963, 1991).

The present inventors have found that the correlation of binding affinity with immunogenicity is an important factor to be considered when evaluating candidate peptides. Thus, by a combination of motif searches and HLA-peptide binding assays, candidates for epitope-based vaccines have been identified. After determining their binding affinity, additional confirmatory work can be performed to select, amongst these vaccine candidates, epitopes with preferred characteristics in terms of antigenicity and

immunogenicity. Various strategies can be utilized to evaluate immunogenicity, including:

1) Evaluation of primary T cell cultures from normal individuals (Wentworth, P. A. *et al.*, *Mol. Immunol.* 32:603, 1995; Celis, E. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:2105, 1994; Tsai, V. *et al.*, *J. Immunol.* 158:1796, 1997; Kawashima, I. *et al.*, *Human Immunol.* 59:1, 1998); This procedure involves the stimulation of PBL from normal subjects with a test peptide in the presence of antigen presenting cells *in vitro* over a period of several weeks. T cells specific for the peptide become activated during this time and are detected using a ^{51}Cr-release assay involving peptide sensitized target cells.

2) Immunization of HLA transgenic mice (Wentworth, P. A. *et al.*, *J. Immunol.* 26:97, 1996; Wentworth, P. A. *et al.*, *Int. Immunol.* 8:651, 1996; Alexander, J. *et al.*, *J. Immunol.* 159:4753, 1997); In this method, peptides in incomplete Freund's adjuvant are administered subcutaneously to HLA transgenic mice. Several weeks following immunization, splenocytes are removed and cultured *in vitro* in the presence of test peptide for approximately one week. Peptide-specific T cells are detected using a ^{51}Cr-release assay involving peptide sensitized target cells and target cells expressing endogenously generated antigen.

3) Demonstration of recall T cell responses from immune individuals who have recovered from infection, and/or from chronically infected patients (Rehermann, B. *et al.*, *J. Exp. Med.* 181:1047, 1995; Doolan, D. L. *et al.*, *Immunity* 7:97, 1997; Bertoni, R. *et al.*, *J. Clin. Invest.* 100:503, 1997; Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997; Diepolder, H. M. *et al.*, *J. Virol.* 71:6011, 1997). In applying this strategy, recall responses were detected by culturing PBL from subjects that had been naturally exposed to the antigen, for instance through infection, and thus had generated an immune response "naturally". PBL from subjects were cultured *in vitro* for 1-2 weeks in the presence of test peptide plus antigen presenting cells (APC) to allow activation of "memory" T cells, as compared to "naive" Tcells. At the end of the culture period, T cell activity is detected using assays for T cell activity including ^{51}Cr release involving peptide-sensitized targets, T cell proliferation or lymphokine release.

The following describes the peptide epitopes and corresponding nucleic acids of the invention.

IV.C. Binding Affinity of Peptide Epitopes for HLA Molecules

As indicated herein, the large degree of HLA polymorphism is an important factor to be taken into account with the epitope-based approach to vaccine development. To address this factor, epitope selection encompassing identification of peptides capable of binding at high or intermediate affinity to multiple HLA molecules is preferably utilized, most preferably these epitopes bind at high or intermediate affinity to two or more allele specific HLA molecules.

CTL-inducing peptides of interest for vaccine compositions preferably include those that have an IC_{50} or binding affinity value for class I HLA molecules of 500 nM or less. HTL-inducing peptides preferably include those that have an IC_{50} or binding affinity value for class II HLA molecules of 1000 nM or less. For example, peptide binding is assessed by testing the capacity of a candidate peptide to bind to a purified HLA molecule *in vitro*. Peptides exhibiting high or intermediate affinity are then considered for further analysis. Selected peptides are tested on other members of the supertype family. In preferred embodiments, peptides that exhibit cross-reactive binding are then used in vaccines or in cellular screening analyses.

As disclosed herein, high HLA binding affinity is correlated with greater immunogenicity. Greater immunogenicity can be manifested in several different ways. Immunogenicity corresponds to whether an immune response is elicited at all, and to the vigor of any particular response. For example, a peptide might elicit an immune response in a diverse array of the population, yet in no instance produce a vigorous response. In accordance with these principles, close to 90% of high binding peptides have been found to be immunogenic, as contrasted with about 50% of the peptides which bind with intermediate affinity. Moreover, higher binding affinity peptides leads to more vigorous immunogenic responses. As a result, less peptide is required to elicit a similar biological effect if a high affinity binding peptide is used. Thus, in preferred embodiments of the invention, high binding epitopes are particularly desired.

The relationship between binding affinity for HLA class I molecules and immunogenicity of discrete peptide epitopes on bound antigens has been determined for the first time in the art by the present inventors. The correlation between binding affinity and immunogenicity was analyzed in two different experimental approaches (Sette, *et al., J. Immunol.* 153:5586-5592, 1994). In the first approach, the immunogenicity of potential epitopes ranging in HLA binding affinity over a 10,000-fold range was analyzed in HLA-A*0201 transgenic mice. In the second approach, the antigenicity of

approximately 100 different hepatitis B virus (HBV)-derived potential epitopes, all carrying A*0201 binding motifs, was assessed by using PBL (peripheral blood lymphocytes) of acute hepatitis patients. Pursuant to these approaches, it was determined that an affinity threshold of approximately 500 nM (preferably an IC_{50} value of 500 nM or less) determines the capacity of a peptide epitope to elicit a CTL response. These data are true for class I binding affinity measurements for naturally processed peptides and for synthesized T cell epitopes. These data also indicate the important role of determinant selection in the shaping of T cell responses.

An affinity threshold associated with immunogenicity in the context of HLA class II DR molecules has also been delineated (Southwood *et al. J. Immunology* 160:3363-3373,1998, and U.S.S.N 60/087192 filed 5/29/98). In order to define a biologically significant threshold of DR binding affinity, a database of the binding affinities of 32 DR-restricted epitopes for their restricting element was compiled. In approximately half of the cases (15 of 32 epitopes), DR restriction was associated with high binding affinities, *i.e.*, binding affinities of with an IC_{50} value of 100 nM or less. In the other half of the cases (16 of 32), DR restriction was associated with intermediate affinity (binding affinities in the 100-1000 nM range). In only one of 32 cases was DR restriction associated with an IC_{50} of 1000 nM or greater. Thus, 1000 nM can be defined as an affinity threshold associated with immunogenicity in the context of DR molecules.

The binding affinity of peptides for HLA molecules can be determined as described in Example 1, below.

IV.D. Peptide Epitope Binding Motifs and Supermotifs

In the past few years evidence has accumulated to demonstrate that a large fraction of HLA class I, and possibly class II molecules can be classified into a relatively few supertypes characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets.

For HLA molecule pocket analyses, the residues comprising the B and F pockets of HLA class I molecules as described in crystallographic studies (Guo, H. C. *et al.*, *Nature* 360:364, 1992; Saper, M. A. , Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991; Madden, D. R., Garboczi, D. N. and Wiley, D. C., *Cell* 75:693, 1993), have been compiled from the database of Parham, *et al.* (Parham, P., Adams, E. J., and Arnett, K. L., *Immunol. Rev.* 143:141, 1995). In these analyses, residues 9, 45, 63, 66, 67, 70, and 99 were considered to make up the B pocket, and to determine the specificity

for the residue in the second position of peptide ligands. Similarly, residues 77, 80, 81, and 116 were considered to determine the specificity of the F pocket, and to determine the specificity for the C-terminal residue of a peptide ligand bound by the HLA molecule.

Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues required for allele-specific binding to HLA molecules have been identified. The presence of these residues correlates with binding affinity for HLA molecules. The identification of motifs and/or supermotifs that correlate with high and intermediate affinity binding is an important issue with respect to the identification of immunogenic peptide epitopes for the inclusion in a vaccine. Kast *et al.* (*J. Immunol.* 152:3904-3912, 1994) have shown that motif-bearing peptides account for 90% of the epitopes that bind to allele-specific HLA class I molecules. In this study all possible peptides of 9 amino acids in length and overlapping by eight amino acids (240 peptides), which cover the entire sequence of the E6 and E7 proteins of human papillomavirus type 16, were evaluated for binding to five allele-specific HLA molecules that are expressed at high frequency among different ethnic groups. This unbiased set of peptides allowed an evaluation of the predictive value of HLA class I motifs. From the set of 240 peptides, 22 peptides were identified that bound to an allele-specific HLA molecules with high or intermediate affinity. Of these 22 peptides, 20, (*i.e.* 91%), were motif-bearing. Thus, this study demonstrates the value of motifs for the identification of peptide epitopes for inclusion in a vaccine: application of motif-based identification techniques eliminates screening of 90% of the potential epitopes.

Such peptide epitopes are identified in the Tables described below. The Tables for the HLA class I epitopes include over 90% of the peptides that will bind to an allele-specific HLA class I molecule with intermediate or high affinity.

Peptides of the present invention may also include epitopes that bind to MHC class II DR molecules. A significant difference between class I and class II HLA molecules is that, although a stringent size restriction exists for peptide binding to class I molecules, a greater degree of heterogeneity in both sizes and binding frame positions of the motif, relative to the N and C termini of the peptide, can be demonstrated for class II peptide ligands. This increased heterogeneity is due to the structure of the class II-binding groove which, unlike its class I counterpart, is open at both ends. Crystallographic analysis of DRB*0101-peptide complexes (*see, e.g.*, Madden, D.R. *Ann. Rev. Immunol.* 13:587, 1995) showed that the residues occupying position 1 and position

6 of peptides complexed with DRB*0101 engage two complementary pockets on the DRBa*0101 molecules, with the P1 position corresponding to the most crucial anchor residue and the deepest hydrophobic pocket. Other studies have also pointed to the P6 position as a crucial anchor residue for binding to various other DR molecules.

Thus, peptides of the present invention are identified by any one of several HLA-specific amino acid motifs(*see, e.g.*, Tables I-III). If the presence of the motif corresponds to the ability to bind several allele-specific HLA antigens it is referred to as a supermotif. The allele-specific HLA molecules that bind to peptides that possess a particular amino acid supermotif are collectively referred to as an HLA "supertype."

The peptide motifs and supermotifs described below provide guidance for the identification and use of peptides in accordance with the invention.

Examples of peptide epitopes bearing the respective supermotif or motif are included in Tables as designated in the description of each motif or supermotif. The Tables include a binding affinity ratio listing for some of the peptide epitopes. The ratio may be converted to IC_{50} by using the following formula: IC_{50} of the standard peptide/ratio = IC_{50} of the test peptide (i.e. the peptide epitope). The IC_{50} values of standard peptides used to determine binding affinities for Class I peptides are shown in Table IV. The IC_{50} values of standard peptides used to determine binding affinities for Class II peptides are shown in Table V. The peptides used as standards for the binding assay are examples of standards; alternative standard peptides can also be used when performing such an analysis.

To obtain the peptide epitope sequences listed in each Table, protein sequence data from twenty HBV strains (HPBADR, HPBADR1CG, HPBADRA, HPBADRC, HPBADRCG, HPBCGADR, HPBVADRM, HPBADW, HPBADW1, HPBADW2, HPBADW3, HPBADWZ, HPBHEPB, HPBVADW2, HPBAYR, HPBV, HPBVAYWC, HPBVAYWCI, NAD HPBVAYWE) were evaluated for the presence of the designated supermotif or motif. Peptide epitopes were also selected on the basis of their conservancy. A criterion for conservancy requires that the entire sequence of a peptide be totally conserved in 75% of the sequences available for a specific protein. The percent conservancy of the selected peptide epitopes is indicated on the Tables. The frequency, *i.e.* the number of strains of the 20 strains in which the peptide sequence was identified, is also shown. The "1st position" column in the Tables designates the amino acid position of the HBV protein that corresponds to the first amino acid residue of the epitope. "Number of amino acids" indicates the number of residues in the epitope sequence.

HLA Class I Motifs Indicative of CTL Inducing Peptide Epitopes:

The primary anchor residues of the HLA class I peptide epitope supermotifs and motifs delineated below are summarized in Table I. The HLA class I motifs set out in Table I(a) are those most particularly relevant to the invention claimed here. Primary and secondary anchor positions are summarized in Table II. Allele-specific HLA molecules that comprise HLA class I supertype families are listed in Table VI.

IV.D1. HLA-A1 supermotif

The HLA-A1 supermotif is characterized by the presence in peptide ligands of a small (T or S) or hydrophobic (L, I, V, M, or F) primary anchor residue in position 2, and an aromatic (Y, F, or W) primary anchor residue at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind to the A1 supermotif (*i.e.*, the HLA-A1 supertype) is comprised of at least A*0101, A*2601, A*2602, A*2501, and A*3201 (*see, e.g.*, DiBrino, M. *et al.*, *J. Immunol.* 151:5930, 1993; DiBrino, M. *et al.*, *J. Immunol.* 152:620, 1994; Kondo, A. *et al.*, *Immunogenetics* 45:249, 1997.). Other allele-specific HLA molecules predicted to be members of the A1 superfamily are shown in Table VI. Peptides binding to each of the individual HLA proteins can be modulated by substitutions at primary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A1 supermotif are set forth on the attached Table VII.

IV.D.2. HLA-A2 supermotif

Primary anchor specificities for allele-specific HLA A2.1 molecules (Falk *et al.*, *Nature* 351:290-296, 1991; Hunt *et al.*, *Science* 255:1261-1263, 1992) and cross-reactive binding within the HLA A2 family (Fruci *et al.*, *Human Immunol.* 38:187-192, 1993; Tanigaki *et al.*, *Human Immunol.* 39:155-162, 1994) have been described. The present inventors have defined additional primary anchor residues that determine cross-reactive binding to multiple allele-specific HLA A2 molecules (Del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995). The HLA-A2 supermotif comprises peptide ligands with L, I, V, M, A, T, or Q as primary anchor residues at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope.

The corresponding family of HLA molecules (*i.e.*, the HLA-A2 supertype that binds these peptides) is comprised of at least: A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, a*0214, A*6802, and A*6901. Other allele-specific HLA molecules predicted to be members of the A2 superfamily are shown in Table VI. As explained in detail below, binding to each of the individual allele-specific HLA molecules can be modulated by substitutions at the primary anchor and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise an A2 supermotif are set forth on the attached Table VIII. The motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.3. HLA-A3 supermotif

The HLA-A3 supermotif is characterized by the presence in peptide ligands of A, L, I, V, M, S, or, T as a primary anchor at position 2, and a positively charged residue, R or K, at the C-terminal position of the epitope (*e.g.*, in position 9 of 9-mers). Exemplary members of the corresponding family of HLA molecules (the HLA-A3 supertype) that bind the A3 supermotif include at least: A*0301, A*1101, A*3101, A*3301, and A*6801. Other allele-specific HLA molecules predicted to be members of the A3 superfamily are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions of amino acids at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A3 supermotif are set forth on the attached Table IX.

IV.D.4. HLA-A24 supermotif

The HLA-A24 supermotif is characterized by the presence in peptide ligands of an aromatic (F, W, or Y) residue as a primary anchor in position 2, and a hydrophobic (Y, F, L, I, V, or M) residue as primary anchor at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind to the A24 supermotif (*i.e.*, the A24 supertype) includes at least A*2402, A*3001, and A*2301. Other allele-specific HLA molecules predicted to be members of the A24 superfamily are shown in Table VI.Peptide binding to each of the allele-specific HLA molecules can be modulated by

substitutions at primary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A24 supermotif are set forth on the attached Table X.

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IV.D.5. HLA-B7 supermotif

The HLA-B7 supermotif is characterized by peptides bearing proline in position 2 as a primary anchor, and a hydrophobic or aliphatic amino acid (L, I, V, M, A, F, W, or Y) as the primary anchor at the C-terminal position of the epitope. The corresponding
10 family of HLA molecules that bind the B7 supermotif (*i.e.*, the HLA-B7 supertype) is comprised of at least twenty six HLA-B proteins including: B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, and B*7801 (*see, e.g.,* Sidney, *et al., J. Immunol.*
15 154:247, 1995; Barber, *et al., Curr. Biol.* 5:179, 1995; Hill, *et al., Nature* 360:434, 1992; Rammensee, *et al., Immunogenetics* 41:178, 1995). Other allele-specific HLA molecules predicted to be members of the B7 superfamily are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions at the primary and/or secondary anchor positions of the
20 peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that contain the B7 supermotif are set forth on the attached Table XI.

IV.D.6. HLA-B27 supermotif

25 The HLA-B27 supermotif is characterized by the presence in peptide ligands of a positively charged (R, H, or K) residue as a primary anchor at position 2, and a hydrophobic (F, Y, L, W, M, I, A, or V) residue as a primary anchor at the C-terminal position of the epitope. Exemplary members of the corresponding family of HLA molecules that bind to the B27 supermotif (*i.e.*, the B27 supertype) include at least
30 B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, and B*7301. Other allele-specific HLA molecules predicted to be members of the B27 superfamily are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B27 supermotif are set forth on the attached Table XII.

IV.D.7. HLA-B44 supermotif

The HLA-B44 supermotif is characterized by the presence in peptide ligands of negatively charged (D or E) residues as a primary anchor in position 2, and hydrophobic residues (F, W, Y, L, I, M, V, or A) as a primary anchor at the C-terminal position of the epitope. Exemplary members of the corresponding family of HLA molecules that bind to the B44 supermotif (*i.e.*, the B44 supertype) include at least: B*1801, B*1802, B*3701, B*4001, B*4002, B*4006, B*4402, B*4403, and B*4006. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary anchor positions; preferably choosing respective residues specified for the supermotif.

IV.D.8. HLA-B58 supermotif

The HLA-B58 supermotif is characterized by the presence in peptide ligands of a small aliphatic residue (A, S, or T) as a primary anchor residue at position 2, and an aromatic or hydrophobic residue (F, W, Y, L, I, V, M, or A) as a primary anchor residue at the C-terminal position of the epitope. Exemplary members of the corresponding family of HLA molecules that bind to the B58 supermotif (*i.e.*, the B58 supertype) include at least: B*1516, B*1517, B*5701, B*5702, and B*5801. Other allele-specific HLA molecules predicted to be members of the B58 superfamily are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B58 supermotif are set forth on the attached Table XIII.

IV.D.9. HLA-B62 supermotif

The HLA-B62 supermotif is characterized by the presence in peptide ligands of the polar aliphatic residue Q or a hydrophobic aliphatic residue (L, V, M, or I) as a primary anchor in position 2, and a hydrophobic residue (F, W, Y, M, I, V, L, or A) as a primary anchor at the C-terminal position of the epitope. Exemplary members of the corresponding family of HLA molecules that bind to the B62 supermotif (*i.e.*, the B62 supertype) include at least: B*1501, B*1502, B*1513, and B5201. Other allele-specific

HLA molecules predicted to be members of the B62 superfamily are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B62 supermotif are set forth on the attached Table XIV.

IV.D.10. HLA-A1 motif

The allele-specific HLA-A1 motif is characterized by the presence in peptide ligands of T, S, or M as a primary anchor residue at position 2 and the presence of Y as a primary anchor residue at the C-terminal position of the epitope. An alternative allele-specific A1 motif (*i.e.*, a "submotif") is characterized by a primary anchor residue at position 3 rather than position 2. This submotif is characterized by the presence of D, E, A, or S as a primary anchor residue in position 3, and a Y as a primary anchor residue at the C-terminal position of the epitope. Peptide binding to HLA A1 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise either A1 motif are set forth on the attached Table XV. Those epitopes comprising T, S, or M at position 2 and Y at the C-terminal position are also included in the listing of HLA-A1 supermotif-bearing peptide epitopes listed in Table VII.

IV.D.11. HLA-A2.1 motif

An allele-specific HLA-A2.1 motif was first determined to be characterized by the presence in peptide ligands of L or M as a primary anchor residue in position 2, and L or V as a primary anchor residue at the C-terminal position of a 9 amino acid epitope (Falk *et al.*, *Nature* 351:290-296, 1991). Furthermore, the A2.1 motif was determined to further comprise an I at position 2 and I or A at the C-terminal position of a nine amino acid peptide (Hunt *et al.*, *Science* 255:1261-1263, March 6, 1992). Additionally, the A2.1 allele-specific motif has been found to comprise a T at the C-terminal position (Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994). Subsequently, the A2.1 allele-specific motif has been defined by the present inventors to additionally comprise V, A, T, or Q as a primary anchor residue at position 2, and M as a primary anchor residue at the C-terminal position of the epitope. Thus, the HLA-A2.1 motif comprises peptide ligands with L, I, V, M, A,

T, or Q as primary anchor residues at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope. The preferred and tolerated residues that characterize the primary anchor positions of the HLA-A2.1 motif are identical to the preferred residues of the A2 supermotif. (for reviews of relevant data, see, *e.g.*, Del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Sidney *et al.*, *Immunol. Today* 17:261-266, 1996; Sette and Sidney, *Curr. Opin. in Immunol.* 10:478-482, 1998). Secondary anchor residues that characterize the A2.1 motif have additionally been defined as disclosed herein. These are disclosed in Table II. Peptide binding to HLA-A2.1 molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise an A2.1 motif are set forth on the attached Table VII. The A2.1 motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.12 HLA-A3 motif

The allele-specific HLA-A3 motif is characterized by the presence in peptide ligands of L, M, V, I, S, A, T, F, C, G, or D as a primary anchor residue at position 2, and the presence of K, Y, R, H, F, or A as a primary anchor residue at the C-terminal position of the epitope. Peptide binding to HLA-A3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A3 motif are set forth on the attached Table XVI. Those peptide epitopes that also comprise the A3 supermotif are also listed in Table IX.

IV.D.13. HLA-A11 motif

The allele-specific HLA-A11 motif is characterized by the presence in peptide ligands of V, T, M, L, I, S, A, G, N, C, D, or F as a primary anchor residue in position 2, and K, R, Y, or H as a primary anchor residue at the C-terminal position of the epitope. Peptide binding to HLA-A11 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A11 motif are set forth on the attached Table XVII; peptide epitopes comprising the A3 allele-specific motif are also present in this Table because of the extensive overlap between the A3 and A11 motif primary anchor specificities. Further, those peptide epitopes that comprise the A3 supermotif are also listed in Table IX.

IV.D.14. HLA-A24 motif

The allele-specific HLA-A24 motif is characterized by the presence in peptide ligands of Y, F, W, or M as a primary anchor residue in position 2, and F, L, I, or W as a primary anchor residue at the C-terminal position of the epitope. Peptide binding to HLA-A24 molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A24 motif are set forth on the attached Table XVIII. These epitopes are also listed in Table X, which sets forth HLA-A24-supermotif-bearing peptide epitopes.

Motifs Indicative of Class II HTL Inducing Peptide Epitopes

The primary and secondary anchor residues of the HLA class II peptide epitope supermotifs and motifs delineated below are summarized in Table III.

IV.D.15. HLA DR-1-4-7 supermotif

Motifs have also been identified for peptides that bind to three common HLA class II allele-specific HLA molecules: HLA DRB1*0401, DRB1*0101, and DRB1*0701. Collectively, the common residues from these motifs delineate the HLA DR-1-4-7 supermotif. Peptides that bind to these DR molecules carry a supermotif characterized by a large aromatic or hydrophobic residue (Y, F, W, L, I, V, or M) as a primary anchor residue in position 1, and a small, non-charged residue (S, T, C, A, P, V, I, L, or M) as a primary anchor residue in position 6 of the epitope. Allele-specific secondary effects and secondary anchors for each of these HLA types have also been identified. These are set forth in Table III. Peptide binding to HLA-DR4, DR1, and/or DR7 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Conserved peptide epitopes (*i.e.* 75% conservancy in the 20 HBV strains used for the analysis), corresponding to a nine residue core comprising the DR-1-4-7 supermotif

(wherein position 1 of the motif is at position 1 of the nine residue core) are set forth in Table XIXa (*see, e.g.*, Madden, Annu. Rev. Immunol. 13:587-622, 1995). Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise a conserved nine residue core, are also shown in section "a" of the Table. Cross-reactive binding data for the exemplary 15-residue supermotif-bearing peptides denoted by a peptide number are shown in Table XIXb.

IV.D.16. HLA DR3 motifs

Two alternative motifs (*i.e.*, submotifs) characterize peptide epitopes that bind to HLA-DR3 molecules. In the first motif (submotif DR3A) a large, hydrophobic residue (L, I, V, M, F, or Y) is present in anchor position 1, and D is present as an anchor at position 4, towards the carboxyl terminus of the epitope.

The alternative DR3 submotif provides for lack of the large, hydrophobic residue at anchor position 1, and/or lack of the negatively charged or amide-like anchor residue at position 4, by the presence of a positive charge at position 6 towards the carboxyl terminus of the epitope. Thus, for the alternative allele-specific DR3 motif (submotif DR3B): L, I, V, M, F, Y, A, or Y is present at anchor position 1; D, N, Q, E, S, or T is present at anchor position 4; and K, R, or H is present at anchor position 6. Peptide binding to HLA-DR3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Conserved peptide epitopes (*i.e.*, sequences that are 75% conservaned in the 20 HBV strains used for the analysis), corresponding to a nine residue core comprising the DR3A submotif (wherein position 1 of the motif is at position 1 of the nine residue core) set forth in Table XXa. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise a conserved nine residue core, are also shown in section "a" of the Table. Table XXb shows binding data of the exemplary DR3 submotif A-bearing peptides denoted by a peptide number.

Conserved peptide epitopes (*i.e.*, 75% conservancy in the 20 HBV strains used for the analysis), corresponding to a nine residue core comprising the DR3B submotif and respective exemplary 15-mer peptides comprising the DR3 submotif-B epitope are set forth in Table XXc. Table XXd shows binding data of the exemplary DR3 submotif B-bearing peptides denoted by a peptide number.

Each of the HLA class I or class II peptide epitopes set out in the Tables herein are deemed singly to be an inventive aspect of this application. Further, it is also an

inventive aspect of this application that each peptide epitope may be used in combination with any other peptide epitope.

IV.E. Enhancing Population Coverage of the Vaccine

Vaccines that have broad population coverage are preferred because they are more commercially viable and generally applicable to the most people. Broad population coverage can be obtained using the peptides of the invention (and nucleic acid compositions that encode such peptides) through selecting peptide epitopes that bind to HLA alleles which, when considered in total, are present in most of the population. Table XXI lists the overall frequencies of the HLA class I supertypes in various ethnicities (Table XXIa) and the combined population coverage achieved by the A2-, A3-, and B7-supertypes (Table XXIb). The A2-, A3-, and B7 supertypes are each present on the average of over 40% in each of these five major ethnic groups. Coverage in excess of 80% is achieved with a combination of these supermotifs. These results suggest that effective and non-ethnically biased population coverage is achieved upon use of a limited number of cross-reactive peptides. Although the population coverage reached with these three main peptide specificities is high, coverage can be expanded to reach 95% population coverage and above, and more easily achieve truly multispecific responses upon use of additional supermotif or allele-specific motif bearing peptides.

The B44-, A1-, and A24-supertypes are present, on average, in a range from 25% to 40%of these major ethnic populations (Table XXIa). While less prevalent overall, the B27-, B58-, and B62 supertypes are each present with a frequency >25% in at least one major ethnic group (Table XXIa). Table XXIb summarizes the estimated combined prevalence in five major ethnic groups of HLA supertypes that have been identified. The incremental coverage obtained by the inclusion of A1,- A24-, and B44-supertypes to the A2, A3, and B7 coverage, or all of the supertypes described herein is shown. By including epitopes from the six most frequent supertypes, an average population coverage of 99% is obtained for five major ethnic groups.

The data presented herein, together with the previous definition of the A2-, A3-, and B7-supertypes, indicates that all antigens, with the possible exception of A29, B8, and B46, can be classified into a total of nine HLA supertypes. Focusing on the six most common supertypes affords population coverage greater than 98% for all major ethnic populations.

IV.F. Immune Response Stimulating Peptide Analogs

Although peptides with suitable cross-reactivity among all alleles of a superfamily are identified by the screening procedures described above, cross-reactivity is not always complete and in such cases procedures to further increase cross-reactivity of peptides can be useful; such procedures can also be used to modify other properties of the peptides. Having established the general rules that govern cross-reactivity of peptides for HLA alleles within a given motif or supermotif, modification (*i.e.,* analoging) of the structure of peptides of particular interest in order to achieve broader (or otherwise modified) HLA binding capacity can be performed. More specifically, peptides which exhibit the broadest cross-reactivity patterns, (both amongst the known T cell epitopes, as well as the more extended set of peptides that contain the appropriate supermotifs), can be produced in accordance with the teachings herein.

The strategy employed utilizes the motifs or supermotifs which correlate with binding to certain HLA molecules. The motifs or supermotifs are defined by having primary anchors, though secondary anchors can also be modified. Analog peptides can be created by substituting amino acids residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or supermotif. Preferred secondary anchor residues of supermotifs and motifs that have been defined for HLA class I and class II binding peptides are shown in Tables II and III, respectively.

For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or members of HLA supertypes that bind to the respective motif or supermotif (Tables II and III). Accordingly, removal of residues that are detrimental to binding can be performed in accordance with the present invention. For example, in the case of the A3 supertype, when all peptides that have such deleterious residues are removed from the population of analyzed peptides, the incidence of cross-reactivity increases from 22% to 37% (see, e.g., Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996). Thus, one strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete one or more of the deleterious residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). An enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, residues associated with high affinity binding to multiple alleles within a superfamily are inserted.

To ensure that an analog peptide, when used as a vaccine, actually elicits a CTL response to the native epitope *in vivo* (or, in the case of class II epitopes, elicits helper T cells that cross-react with the wild type peptides), the analog peptide may be used to immunize T cells *in vitro* from individuals of the appropriate HLA allele. Thereafter, the immunized cells' capacity to induce lysis of wild type peptide sensitized target cells is evaluated. It will be desirable to use as antigen presenting cells, cells that have been either infected, or transfected with the appropriate genes, or, in the cae of class II epitopes only, cells that have been pusled with whole protein antigens, to establish whether endogenously produced antigen is also recognized by the relevant T cells.

Another embodiment of the invention to ensure adequate numbers of cross-reactive cellular binders is to create analogs of weak binding peptides. Class I peptides exhibiting binding affinities of 500-50000nM, and carrying an acceptable but suboptimal primary anchor residue at one or both positions can be "fixed" by substituting preferred anchor residues in accordance with the respective supertype. The analog peptides can then be tested for crossbinding activity.

Another embodiment for generating effective peptide analogs involves the substitution of residues that have an adverse impact on peptide stability or solubility in a liquid environment. This substitution may occur at any position of the peptide epitope. For example, a cysteine (C) can be substituted out in favor of α-amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substituting α-amino butyric acid for C not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances (Review: A. Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999). Substitution of cysteine with α-amino butyric acid may occur at any residue of a peptide epitope, i.e. at either anchor or non-anchor positions.

In general, CTL and HTL responses are not directed against all possible epitopes. Rather, they are restricted to a few immunodominant determinants (Zinkernagel, *et al., Adv. Immunol.* 27:5159, 1979; Bennink, *et al., J. Exp. Med.* 168:19351939, 1988; Rawle, *et al., J. Immunol.* 146:3977-3984, 1991). It has been recognized that immunodominance (Benacerraf, *et al., Science* 175:273-279, 1972) could be explained by either the ability of a given epitope to selectively bind a particular HLA protein (determinant selection theory) (Vitiello, *et al., J. Immunol.* 131:1635, 1983); Rosenthal, *et al., Nature* 267:156-158, 1977), or being selectively recognized by the existing TCR (T cell receptor)

specificities (repertoire theory) (Klein, J., IMMUNOLOGY, THE SCIENCE OF SELFNONSELF DISCRIMINATION, John Wiley & Sons, New York, pp. 270-310, 1982). It has been demonstrated that additional factors, mostly linked to processing events, can also play a key role in dictating, beyond strict immunogenicity, which of the many potential determinants will be presented as immunodominant (Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993).

The concept of dominance and subdominance is relevant to immunotherapy of both infectious diseases and cancer. For example, in the course of chronic viral disease, recruitment of subdominant epitopes can be important for successful clearance of the infection, especially if dominant CTL or HTL specificities have been inactivated by functional tolerance, suppression, mutation of viruses and other mechanisms (Franco, *et al., Curr. Opin. Immunol.* 7:524-531, (1995)). In the case of cancer and tumor antigens, CTLs recognizing at least some of the highest binding affinity peptides might be functionally inactivated. Lower binding affinity peptides are preferentially recognized at these times.

In particular, it has been noted that a significant number of epitopes derived from known non-viral tumor associated antigens (TAA) bind HLA class I with intermediate affinity (IC_{50} in the 50-500 nM range). For example, it has been found that 8 of 15 known TAA peptides recognized by tumor infiltrating lymphocytes (TIL) or CTL bound in the 50-500 nM range. (These data are in contrast with estimates that 90% of known viral antigens that were recognized as peptides bound HLA with IC_{50} of 50 nM or less, while only approximately 10% bound in the 50-500 nM range (Sette, *et al., J. Immunol.*, 153:558-5592 (1994)). In the cancer setting this phenomenon is probably due to elimination, or functional inhibition of the CTL recognizing several of the highest binding peptides, presumably because of T cell tolerization events.

Without intending to be bound by theory, it is believed that because T cells to dominant epitopes may have been clonally deleted, selecting subdominant epitopes may allow extant T cells to be recruited, which will then lead to a therapeutic response. However, the binding of HLA molecules to subdominant epitopes is often less vigorous than to dominant ones. Accordingly, there is a need to be able to modulate the binding affinity of particular immunogenic epitopes for one or more HLA molecules, and thereby to modulate the immune response elicited by the peptide. Thus, a need exists to prepare analog peptides which elicit a more vigorous response. This ability would greatly enhance the usefulness of peptide-based vaccines and therapeutic agents.

Representative analog peptides are set forth in Table XXII. The Table indicates the length and sequence of the analog peptide as well as the motif or supermotif, if appropriate. The information in the "Fixed Nomenclature" column indicates the residues substituted at the indicated position numbers for the respective analog.

IV.G. Computer Screening of Protein Sequences from Disease-Related Antigens for Supermotif or Motif Containing Peptides

Computer programs that allow the rapid screening of protein sequences for the occurrence of the subject supermotifs or motifs are encompassed by the present invention; as are programs that permit the generation of analog peptides. These programs are implemented to analyze any identified amino acid sequence or operate on an unknown sequence and simultaneously determine the sequence and identify motif-bearing epitopes thereof; analogs can be simultaneously determined as well. Generally, the identified sequences will be from a pathogenic organism or a tumor-associated peptide. For example, the target molecules considered herein include all of the HBV proteins (e.g. surface, core, polymerase, and X).

In cases where the sequence of multiple variants of the same target protein are available, peptides may also be selected on the basis of their conservancy. A presently preferred criterion for conservancy defines that the entire sequence of a peptide be totally conserved in 75% of the sequences evaluated for a specific protein; this definition of conservancy has been employed herein.

It is important that the selection criteria utilized for prediction of peptide binding are as accurate as possible, to correlate most efficiently with actual binding. Prediction of peptides that bind, for example, to HLA-A*0201, on the basis of the presence of the appropriate primary anchors, is positive at about a 30% rate (Ruppert, J. *et al. Cell* 74:929, 1993). However, by analyzing an extensive peptide-HLA binding database, the present inventors have developed a number of allele specific polynomial algorithms that dramatically increase the predictive value over identification on the basis of the presence of primary anchor residues alone. These algorithms take into account not only the presence or absence of the correct primary anchors, but also consider the positive or deleterious presence of secondary anchor residues (to account for the impact of different amino acids at different positions). The algorithms are essentially based on the premise

that the overall affinity (or ΔG) of peptide-HLA interactions can be approximated as a linear polynomial function of the type:

$$\Delta G = a_{1i} \times a_{2i} \times a_{3i} \ldots \times a_{ni}$$

where a_{ij} is a coefficient that represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. An important assumption of this method is that the effects at each position are essentially independent of each other. This assumption is justified by studies that demonstrated that peptides are bound to HLA molecules and recognized by T cells in essentially an extended conformation. Derivation of specific algorithm coefficients has been described in Gulukota *et al.* (Gulukota, K. *et al.*, *J.Mol.Biol.* 267:1258, 1997).

Additional methods to identify preferred peptide sequences, which also make use of specific motifs, include the use of neural networks and molecular modeling programs (Gulukota, K. *et al.*, *J.Mol.Biol.* 267:1258, 1997; Milik *et al.*, *Nature Biotechnology* 16:753, 1998; Altuvia *et al.*, *Hum. Immunol.* 58:1, 1997; Altuvia et al, *J. Mol. Biol.* 249:244, 1995; Buus, S. *Curr. Opin. Immunol.* 11:209-213, 1999; Brusic, V. *et al.*, *Bioinformatics* 14:121-130, 1998).

For example, it has been shown that in sets of A*0201 motif peptides, 69% of the peptides containing at least one preferred secondary anchor residue while avoiding the presence of any deleterious secondary anchor residues, will bind A*0201 with an IC_{50} less than 500 nM (Ruppert, J. *et al. Cell* 74:929, 1993). These algorithms are also flexible in that cut-off scores may be adjusted to select sets of peptides with greater or lower predicted binding properties, as desired.

In utilizing computer screening to identify peptide epitopes, all protein sequence or translated sequence may be analyzed using software developed to search for motifs, for example the "FINDPATTERNS' program (Devereux, *et al. Nucl. Acids Res.* 12:387-395, 1984) or MotifSearch 1.4 software program (D. Brown, San Diego, CA) to identify potential peptide sequences containing appropriate HLA binding motifs. As appreciated by one of ordinary skill in the art a large array of software and hardware options are available which can be employed to implement the motifs of the invention relative to known or unknown peptide sequences. The identified peptides will then be scored using customized polynomial algorithms to predict their capacity to bind specific HLA class I or class II alleles.

In accordance with the procedures described above, HBV peptides and analogs thereof that are able to bind HLA supertype groups or allele-specific HLA molecules have been identified (Tables VII-XX; Table XXII).

IV.H. Preparation of Peptide Epitopes

Peptides in accordance with the invention can be prepared synthetically, by recombinant DNA technology, or from natural sources such as native tumors or pathogenic organisms. Peptide epitopes may be synthesized individually or as polyepitopic peptides. Although the peptide will preferably be substantially free of other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptides may be synthetically conjugated to native fragments or particles.

The peptides in accordance with the invention can be a variety of lengths, and either in their neutral (uncharged) forms or in forms which are salts. The peptides in accordance with the invention are either free of modifications such as glycosylation, side chain oxidation, or phosphorylation; or they contain these modifications, subject to the condition that modifications do not destroy the biological activity of the peptides as described herein.

Desirably, the peptide will be as small as possible while still maintaining substantially all of the biological activity of the large peptide. When possible, it may be desirable to optimize HLA class I binding peptides of the invention to a length of about 8 to about 13 amino acid residues, preferably 9 to 10. HLA class II binding peptides may be optimized to a length of about 6 to about 25 amino acids in length, preferably to between about 13 and about 20 residues. Preferably, the peptides are commensurate in size with endogenously processed pathogen-derived peptides or tumor cell peptides that are bound to the relevant HLA molecules. Moreover, the identification and preparation of peptides of other lengths can be carried out using the techniques described herein (e.g., the disclosures regarding primary and secondary anchor positions). However, it is also preferred to identify a larger region of a native peptide that encompasses one and preferably two or more epitopes in accordance with the invention. This sequence is selected on the basis that it contains the greatest number of epitopes per amino acid length. It is to be appreciated that epitopes can be present in a frame-shifted manner, e.g. a 10 amino acid long peptide could contain two 9 amino acid long epitopes and one 10 amino acid long epitope; each epitope can be exposed and bound by an HLA molecule upon administration of a plurality of such peptides. This larger, preferably multi-epitopic,

peptide can then be generated synthetically, recombinantly, or via cleavage from the native source.

The peptides of the invention can be prepared in a wide variety of ways. For the preferred relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart & Young, SOLID PHASE PEPTIDE SYNTHESIS, 2D. ED., Pierce Chemical Co. (1984). Further, individual peptides may be joined using chemical ligation to produce larger peptides.

Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes an immunogenic peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook *et al.,* MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989). Thus, recombinant polypeptides which comprise one or more peptide sequences of the invention can be used to present the appropriate T cell epitope.

As the nucleotide coding sequence for peptides of the preferred lengths contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, *et al., J. Am. Chem. Soc.* 103:3185 (1981) modification can be made simply by substituting the appropriate and desired nucleic acid base(s) for those that encode the native peptide sequence. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems are now available. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts. Of course, yeast, insect or mammalian cell hosts may also be used, employing suitable vectors and control sequences.

IV.I. Assays to Detect T-Cell Responses

Once HLA binding peptides are identified, they can be tested for the ability to elicit a T-cell response. The preparation and evaluation of motif-bearing peptides are described in PCT publications WO 94/20127 and WO 94/03205. Briefly, peptides comprising epitopes from a particular antigen are synthesized and tested for their ability to bind to the appropriate HLA proteins in assays using, for example, purified HLA class I molecules and radioiodonated peptides and/or cells expressing empty class I molecules (which lack peptide in their receptor) by, for instance, immunofluorescent staining and flow microfluorimetry, peptide-dependent class I assembly assays, and inhibition of CTL recognition by peptide competition. Those peptides that bind to the class I molecule are further evaluated for their ability to serve as targets for CTLs derived from infected or immunized individuals, as well as for their capacity to induce primary *in vitro* or *in vivo* CTL responses that can give rise to CTL populations capable of reacting with selected target cells associated with a disease. Corresponding assays are used for evaluation of HLA class II binding peptides.

Conventional assays utilized to detect CTL responses include proliferation assays, lymphokine secretion assays, direct cytotoxicity assays, and limiting dilution assays. For example, antigen-presenting cells that have been incubated with a peptide can be assayed for the ability to induce CTL responses in responder cell populations. Antigen-presenting cells can be normal cells such as peripheral blood mononuclear cells or dendritic cells. Alternatively, mutant mammalian cell lines that are deficient in their ability to load class I molecules with internally processed peptides and that have been transfected with the appropriate human class I gene may be used to test for the capacity of the peptide to induce *in vitro* primary CTL responses.

Peripheral blood lymphocytes may be used as the responder cell source of CTL precursors. The appropriate antigen-presenting cells are incubated with peptide and the peptide-loaded antigen-presenting cells are then incubated with the responder cell population under optimized culture conditions. Positive CTL activation can be determined by assaying the culture for the presence of CTLs that kill radio-labeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed forms of the HBV antigen from which the peptide sequence was derived.

More recently, a method has also been devised which allows direct quantification of antigen-specific T cells by staining with Fluorescein-labelled HLA tetrameric complexes (Altman, J. D. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:10330, 1993; Altman, J.

D. *et al.*, *Science* 274:94, 1996). Other relatively recent technical developments include staining for intracellular lymphokines, and interferon release assays or ELISPOT assays. Tetramer staining, intracellular lymphokine staining and ELISPOT assays all appear to be at least 10-fold more sensitive than more conventional assays (Lalvani, A. *et al.*, *J. Exp. Med.* 186:859, 1997; Dunbar, P. R. *et al.*, *Curr. Biol.* 8:413, 1998; Murali-Krishna, K. *et al.*, *Immunity* 8:177, 1998).

HTL activation may also be assessed using such techniques as T cell proliferation and secretion of lymphokines, e.g. IL-2.

Alternatively, immunization of HLA transgenic mice can be used to determine immunogenicity of peptide epitopes. Several transgenic mouse models including mice with human A2.1, A11, and B7 alleles have been characterized and others (e.g., transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed. Additional transgenic mouse models with other HLA alleles may be generated as necessary. Mice may be immunized with peptides emulsified in Incomplete Freund's Adjuvant and the resulting T cells tested for their capacity to recognize peptide-pulsed target cells and target cells transfected with appropriate genes. CTL responses may be analyzed using cytotoxicity assays described above. Similarly, HTL responses may be analyzed using such assays as T cell proliferation or secretion of lymphokines.

Immunogenic peptide epitopes are set out in Table XXIII.

IV.J. Use of Peptide Epitopes as Diagnostic Agents and for Evaluating Immune Responses

HLA class I and class II binding peptides as described herein can be used, in one embodiment of the invention, as reagents to evaluate an immune response. The immune response to be evaluated may be induced by using as an immunogen any agent that would potentially result in the production of antigen-specific CTLs or HTLs to the peptide epitope(s) to be employed as the reagent. The peptide reagent is not used as the immunogen.

For example, a peptide of the invention may be used in a tetramer staining assay to assess peripheral blood mononuclear cells for the presence of antigen-specific CTLs following exposure to a pathogen or immunogen. The HLA-tetrameric complex is used to directly visualize antigen-specific CTLs (*see, e.g.*, Ogg *et al.* Science 279:2103-2106, 1998; and Altman *et al.* Science 174:94-96, 1996) and determine the frequency of the

antigen-specific CTL population in a sample of peripheral blood mononuclear cells. A tetramer reagent using a peptide of the invention may be generated as follows: A peptide that binds to an allele-specific HLA molecules, or supertype molecules, is refolded in the presence of the corresponding HLA heavy chain and β_2-microglobulin to generate a trimolecular complex. The complex is biotinylated at the carboxyl terminal end of the heavy chain at a site that was previously engineered into the protein. Tetramer formation is then induced by the addition of streptavidin. By means of fluorescently labeled streptavidin, the tetramer can be used to stain antigen-specific cells. The cells may then be identified, for example, by flow cytometry. Such an analysis may be used for diagnostic or prognostic purposes.

Peptides of the invention may also be used as reagents to evaluate immune recall responses. (*see, e.g.*, Bertoni *et al. J. Clin. Invest.* 100:503-513, 1997 and Penna *et al. J. Exp. Med.* 174:1565-1570, 1991.) For example, patient PBC samples from individuals with acute hepatitis B or who have recently recovered from acute hepatitis B may be analyzed for the presence of HBV antigen-specific CTLs using HBV-specific peptides. A blood sample containing mononuclear cells may be evaluated by cultivating the PBCs and stimulating the cells with a peptide of the invention. After an appropriate cultivation period, the expanded cell population may be analyzed for cytotoxic activity.

The peptides may also be used as reagents to evaluate the efficacy of a vaccine. PBMCs obtained from a patient vaccinated with an immunogen may be analyzed using, for example, either of the methods described above. A patient is HLA typed, and appropriate peptide reagents that recognize allele-specific molecules present in that patient may be selected for the analysis. The immunogenicity of the vaccine will be indicated by the presence of HBV epitope-specific CTLs in the PBMC sample.

The peptides of the invention may also be used to make antibodies using techniques well known in the art (*see, e.g.*, *CURRENT PROTOCOLS IN IMMUNOLOGY*, Wiley/Greene, NY; and *Antibodies A Laboratory Mnaual*, Harlow and Lane, Cold Spring Harbor Laboratory Press, 1989). Such antibodies may be useful as reagents to diagnose HBV infection.

IV.K. Vaccine Compositions

Vaccines that contain an immunogenically effective amount of one or more peptides as described herein are a further embodiment of the invention. Once

appropriately immunogenic epitopes have been defined, they can be sorted and delivered by various means, herein referred to as "vaccine" compositions. Such vaccine compositions can include, for example, lipopeptides (Vitiello, A. *et al.*, *J. Clin. Invest.* 95:341, 1995), peptides compositions encapsulated in poly(DL-lactide-co-glycolide) (PLG) microspheres (see, e.g., Eldridge, *et al. Molec. Immunol.* 28:287-294, 1991: *Alonso et al.* Vaccine 12:299-306, 1994; Jones *et al. Vaccine* 13:675-681, 1995), peptide compositions encapsulated in immune stimulating complexes (ISCOMS) (see, e.g., Takahashi *et al.* Nature 344:873-875, 1990; Hu *et al.* Clin Exp Immunol. 113:235-243, 1998), multiple antigen peptide systems (MAPs) (see e.g., Tam, J. P., Proc. Natl. Acad. Sci. U.S.A. 85:5409-5413, 1988; Tam, J.P., J. Immunol. Methods 196:17-32, 1996), viral delivery vectors (Perkus, M. E. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 379, 1996; Chakrabarti, S. *et al.*, *Nature* 320:535, 1986; Hu, S. L. *et al.*, *Nature* 320:537, 1986; Kieny, M.-P. *et al.*, *AIDS Bio/Technology* 4:790, 1986; Top, F. H. *et al.*, *J. Infect. Dis.* 124:148, 1971; Chanda, P. K. *et al.*, *Virology* 175:535, 1990), particles of viral or synthetic origin (Kofler, N. *et al.*, *J. Immunol. Methods.* 192:25, 1996; Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993; Falo, L. D., Jr. *et al.*, *Nature Med.* 7:649, 1995), adjuvants (Warren, H. S., Vogel, F. R., and Chedid, L. A. *Annu. Rev. Immunol.* 4:369, 1986; Gupta, R. K. *et al.*, *Vaccine* 11:293, 1993), liposomes (Reddy, R. *et al.*, *J. Immunol.* 148:1585, 1992; Rock, K. L., *Immunol. Today* 17:131, 1996), or, naked or particle absorbed cDNA (Ulmer, J. B. *et al.*, *Science* 259:1745, 1993; Robinson, H. L., Hunt, L. A., and Webster, R. G., *Vaccine* 11:957, 1993; Shiver, J. W. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 423, 1996; Cease, K. B., and Berzofsky, J. A., *Annu. Rev. Immunol.* 12:923, 1994 and Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993). Toxin-targeted delivery technologies, also known as receptor mediated targeting, such as those of Avant Immunotherapeutics, Inc. (Needham, Massachusetts) may also be used.

Furthermore, vaccines in accordance with the invention encompass compositions of one or more of the claimed peptide(s) that can be introduced into a host, including humans, linked to its own carrier, or as a homopolymer or heteropolymer of active peptide units. Such a polymer has the advantage of increased immunological reaction and, where different peptides are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the pathogenic organism or tumor-related peptide targetted for an immune response.

Furthermore, useful carriers that can be used with vaccines of the invention are well known in the art, and include, *e.g.,* thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly L-lysine, poly L-glutamic acid, influenza, hepatitis B virus core protein, and the like. The vaccines can contain a physiologically tolerable (i.e., acceptable) diluent such as water, or saline, preferably phosphate buffered saline. The vaccines also typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are examples of materials well known in the art. Additionally, as disclosed herein, CTL responses can be primed by conjugating peptides of the invention to lipids, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P_3CSS).

As disclosed in greater detail herein, upon immunization with a peptide composition in accordance with the invention, via injection, aerosol, oral, transdermal, transmucosal, intrapleural, intrathecal, or other suitable routes, the immune system of the host responds to the vaccine by producing large amounts of CTLs specific for the desired antigen. Consequently, the host becomes at least partially immune to later infection, or at least partially resistant to developing an ongoing chronic infection, or derives at least some therapeutic benefit when the antigen was tumor-associated.

In some instances it may be desirable to combine the class I peptide vaccines of the invention with vaccines which induce or facilitate neutralizing antibody responses to the target antigen of interest, particularly to viral envelope antigens. A preferred embodiment of such a composition comprises class I and class II epitopes in accordance with the invention. An alternative embodiment of such a composition comprises a class I and/or class II epitope in accordance with the invention, along with a PADRE™ (Epimmune, San Diego, CA) molecule (described in the related U.S.S.N. 08/485,218, which is a CIP of U.S.S.N. 08/305,871, now U.S. Patent Number 5,736,142, which is a CIP of abandoned application U.S.S.N. 08/121,101.) Furthermore, any of these embodiments can be administered as a nucleic acid mediated modality.

For therapeutic or immunization purposes, the peptides of the invention can also be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into an acutely or chronically infected host or into a non-infected host, the recombinant vaccinia virus expresses the immunogenic peptide, and

thereby elicits a host CTL and/or HTL response. Vaccinia vectors and methods useful in immunization protocols are described in, *e.g.*, U.S. Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover, *et al. Nature* 351:456-460 (1991). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, e.g. adeno and adeno-associated virus vectors, retroviral vectors, *Salmonella typhi* vectors, detoxified anthrax toxin vectors, and the like, will be apparent to those skilled in the art from the description herein.

Antigenic peptides are used to elicit a CTL and/or HTL response *ex vivo*, as well. The resulting CTL or HTL cells, can be used to treat chronic infections, or tumors in patients that do not respond to other conventional forms of therapy, or will not respond to a therapeutic vaccine peptide or nucleic acid in accordance with the invention. *Ex vivo* CTL or HTL responses to a particular pathogen (infectious agent or tumor antigen) are induced by incubating in tissue culture the patient's CTL or HTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptide. After an appropriate incubation time (typically about 14 weeks), in which the precursor cells are activated, mature and expand into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cell (an infected cell or a tumor cell). Transfected dendritic cells may also be used as antigen presenting cells. Alternatively, dendritic cells are transfected, *e.g.*, with a minigene construct in accordance with the invention, in order to elicit immune responses. Minigenes will be discussed in greater detail in a following section.

DNA or RNA encoding one or more of the peptides of the invention can also be administered to a patient. This approach is described, for instance, in Wolff *et. al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720; and in more detail below. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivicaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") delivery.

Preferably, the following principles are utilized when selecting an array of epitopes for inclusion in a polyepitopic composition, or for selecting epitopes to be included in a vaccine composition and/or to be encoded by a minigene. It is preferred that each of the following principles are balanced in order to make the selection.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with HBV clearance. For HLA Class I this includes 3-4 epitopes that come from at least one antigen of HBV. In other words, it has been observed that in patients who spontaneously clear HBV, that they had generated an immune response to at least 3 epitopes on at least one HBV antigen. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one HBV antigen (see *e.g.*, Rosenberg *et al. Science* 278:1447-1450).

2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, or for Class II an IC_{50} of 1000 nM or less.

3.) Sufficient supermotif bearing peptides, or a sufficient array of allele-specific motif bearing peptides, are selected to give broad population coverage. For example, it is preferable to have at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess population coverage.

4.) When selecting epitopes from cancer-related antigens it is often preferred to select analogs. When selecting epitopes for infectious disease-related antigens it is often preferable to select native epitopes. Therefore, of particular relevance for infectious disease vaccines (but for cancer-related vaccines as well), are epitopes referred to as "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A peptide comprising "transcendent nested epitopes" is a peptide that has both HLA class I and HLA class II epitopes in it.

When providing nested epitopes, it is preferable to provide a sequence that has the greatest number of epitopes per provided sequence. A limitation on this principle is to avoid providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a longer peptide sequence, such as a sequence comprising nested epitopes, it is important to screen the sequence in order to insure that it does not have pathological or other deleterious biological properties.

5.) When creating a minigene, as disclosed in greater detail in the following section, an objective is to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same as those employed when selecting a peptide comprising nested epitopes. Thus, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide

encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is an actual binding epitope, as predicted, e.g., by motif analysis. Junctional epitopes are to be avoided because the recipient may generate an immune response to that epitope. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

IV.K.1. Minigene Vaccines

A growing body of experimental evidence demonstrates that a number of different approaches are available which allow simultaneous delivery of multiple epitopes. Nucleic acids encoding the peptides of the invention are a particularly useful embodiment of the invention. Epitopes for inclusion in a minigene are preferably selected according to the guidelines set forth in the previous section. A preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding a peptide comprising one or multiple epitopes of the invention. The use of multi-epitope minigenes is described below and in, *e.g.* An, L. and Whitton, J. L., *J. Virol.* 71:2292, 1997; Thomson, S. A. *et al.*, *J. Immunol.* 157:822, 1996; Whitton, J. L. *et al.*, *J. Virol.* 67:348, 1993; Hanke, R. *et al.*, *Vaccine* 16:426, 1998. For example, a multi-epitope DNA plasmid encoding nine dominant HLA-A*0201- and A11-restricted epitopes derived from the polymerase, envelope, and core proteins of HBV and HIV, the PADRE™ universal helper T cell (HTL) epitope, and an endoplasmic reticulum-translocating signal sequence was engineered. Immunization of HLA transgenic mice with this plasmid construct resulted in strong CTL induction responses against the nine epitopes tested, similar to those observed with a lipopeptide of known immunogenicity in humans, and significantly greater than immunization in oil-based adjuvants. Moreover, the immunogenicity of DNA-encoded epitopes *in vivo* correlated with the *in vitro* responses of specific CTL lines against target cells transfected with the DNA plasmid. Thus, these data show that the minigene served to both: 1.) generate a CTL response and 2.) that the induced CTLs recognized cells expressing the encoded epitopes.

For example, to create a DNA sequence encoding the selected epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes may be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences may be directly adjoined, so that

when translated, a continuous polypeptide sequence is created. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that could be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a ubiquitination signal sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes may be improved by including synthetic (*e.g.* poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes; these larger peptides comprising the epitope(s) are within the scope of the invention.

The minigene sequence may be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) may be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

Standard regulatory sequences well known to those of skill in the art are preferably included in the vector to ensure expression in the target cells. Several vector elements are desirable: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (*e.g.* ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.,* the human cytomegalovirus (hCMV) promoter. See, *e.g.*, U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells may also be considered for increasing minigene expression.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are

confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

In addition, immunostimulatory sequences (ISSs or CpGs) appear to play a role in the immunogenicity of DNA vaccines. These sequences may be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity.

In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (*e.g.*, IL-2, IL-12, GM-CSF), cytokine-inducing molecules (*e.g.*, LeIF) or costimulatory molecules. Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving CTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (*e.g.* TGF-β) may be beneficial in certain diseases).

Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffer saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids can also be used in the formulation (*see, e.g.*, as described by WO 93/24640; Mannino & Gould-Fogerite, *BioTechniques* 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, *et*

al., Proc. Nat'l Acad. Sci. USA 84:7413 (1987). In addition, glycolipids, fusogenic liposomes, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

Target cell sensitization can be used as a functional assay for expression and HLA class I presentation of minigene-encoded CTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 (^{51}Cr) labeled and used as target cells for epitope-specific CTL lines; cytolysis, detected by ^{51}Cr release, indicates both production of, and HLA presentation of, minigene-encoded CTL epitopes.

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (*e.g.*, IM for DNA in PBS, intraperitoneal (IP) for lipid-complexed DNA). Twenty-one days after immunization, splenocytes are harvested and restimulated for 1 week in the presence of peptides encoding each epitope being tested. Thereafter, for CTL effector cells, assays are conducted for cytolysis of peptide-loaded, ^{51}Cr labeled target cells using standard techniques. Lysis of target cells sensitized by HLA loading of peptides corresponding to minigene-encoded epitopes demonstrates DNA vaccine function for *in vivo* induction of CTLs. Immunogenicity of HTL epitopes is evaluated in transgenic mice in an analogous manner.

Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

IV.K.2. Combinations of CTL Peptides with Helper Pepides

The peptides of the present invention, or analogs thereof, which have immunostimulatory activity may be modified to provide desired attributes, such as improved serum half life, or to enhance immunogenicity.

For instance, the ability of the peptides to induce CTL activity can be enhanced by linking the peptide to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. The use of T helper epitopes in conjunction with CTL epitopes to enhance immunogenicity is illustrated, for example, in U.S.S.N. 08/820360, U.S.S.N. 08/197,484, U.S.S.N. 08/464,234, U.S.S.N. 08/464,496, U.S.S.N. 08/464,031, abandoned U.S.S.N. 08/464,433, and U.S.S.N. 08/461,603.

Particularly preferred CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, *e.g.*, Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues. Alternatively, the CTL peptide may be linked to the T helper peptide without a spacer.

The CTL peptide epitope may be linked to the HTL peptide epitope either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the CTL epitope or the HTL peptide may be acylated. The HTL peptide epitopes used in the invention can be modified in the same manner as CTL peptides. For instance, they may be modified to include D-amino acids or be conjugated to other molecules such as lipids, proteins, sugars and the like. Exemplary T helper peptides include tetanus toxoid 830-843, influenza 307-319, and malarial circumsporozoite 382-398 and 378-389.

In certain embodiments, the T helper peptide is one that is recognized by T helper cells present in the majority of the population. This can be accomplished by selecting amino acid sequences that bind to many, most, or all of the HLA class II molecules. These are known as "loosely HLA-restricted" or "promiscuous" T helper sequences. Examples of amino acid sequences that are promiscuous include sequences from antigens such as tetanus toxoid at positions 830-843 (QYIKANSKFIGITE), *Plasmodium falciparum* CS protein at positions 378-398 (DIEKKIAKMEKASSVFNVVNS), and

Streptococcus 18kD protein at positions 116 (GAVDSILGGVATYGAA). Other examples include peptides bearing a DR 1-4-7 supermotif, or either of the DR3 motifs.

Alternatively, it is possible to prepare synthetic peptides capable of stimulating T helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in nature (*see, e.g.*, PCT publication WO 95/07707). These synthetic compounds called Pan-DR-binding epitopes (*e.g.*, PADRE™, Epimmune, Inc., San Diego, CA) are designed on the basis of their binding activity to most HLA-DR (human HLA class II) molecules. For instance, a pan-DR-binding epitope peptide having the formula: aKXVWANTLKAAa, where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and a is either D-alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals, regardless of their HLA type.

HTL peptide epitopes can also be modified to alter their biological properties. For example, peptides comprising HTL epitopes can contain D-amino acids to increase their resistance to proteases and thus extend their serum half-life. Also, the epitope peptides of the invention can be conjugated to other molecules such as lipids, proteins or sugars, or any other synthetic compounds, to increase their biological activity. Specifically, the T helper peptide can be conjugated to one or more palmitic acid chains at either the amino or carboxyl termini.

In some embodiments it may be desirable to include in the pharmaceutical compositions of the invention at least one component which primes cytotoxic T lymphocytes. Lipids have been identified as agents capable of priming CTL *in vivo* against viral antigens. For example, palmitic acid residues can be attached to the ε-and α-amino groups of a lysine residue and then linked, *e.g.*, via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic peptide. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant, *e.g.*, incomplete Freund's adjuvant. In a preferred embodiment, a particularly effective immunogenic comprises palmitic acid attached to ε- and α- amino groups of Lys, which is attached via linkage, *e.g.*, Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P_3CSS) can be used to prime virus specific CTL when covalently attached to an appropriate peptide. (*See*, Deres, *et al.*,

Nature 342:561, 1989). Peptides of the invention can be coupled to P_3CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Moreover, because the induction of neutralizing antibodies can also be primed with P_3CSS-conjugated epitopes, two such compositions can be combined to more effectively elicit both humoral and cell-mediated responses to infection.

In addition, additional amino acids can be added to the termini of a peptide to provide for ease of linking peptides one to another, for coupling to a carrier support, or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide, particularly class I peptides. However, it is to be noted that modification at the carboxyl terminus of a CTL epitope may, in some cases, alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH_2 acylation, *e.g.*, by alkanoyl (C_1-C_{20}) or thioglycolyl acetylation, terminal-carboxyl amidation, *e.g.*, ammonia, methylamine, *etc.* In some instances these modifications may provide sites for linking to a support or other molecule.

IV.L. Administration of Vaccines for Therapeutic or Prophylactic Purposes

The peptides of the present invention and pharmaceutical and vaccine compositions of the invention are useful for administration to mammals, particularly humans, to treat and/or prevent HBV infection. Vaccine compositions containing the peptides of the invention are administered to a patient susceptible to or otherwise at risk for HBV infection to elicit an immune response against HBV antigens and thus enhance the patient's own immune response capabilities. In therapeutic applications, compositions are administered to a patient in an amount sufficient to elicit an effective CTL response to the virus antigen and to cure or at least partially arrest or slow symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, *e.g.*, the particular composition administered, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

The vaccine compositions of the invention may also be used purely as prophylactic agents. Vaccine compositions containing the peptide epitopes of the

invention are administered to a patient susceptible to, or otherwise at risk for, HBV infection to elicit an immune response against HBV antigens and thus enhance the patient's own immune response capabilities following exposure to HBV. Generally the dosage for an initial prophylactic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg. Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. This is followed by boosting dosages of between about 1.0 μg to about 50,000 μg of peptide administered at defined intervals from about four weeks to six months after the initial administration of vaccine. The immunogenicity of the vaccine may be assessed by measuring the specific activity of CTL and/or HTL obtained from a sample of the patient's blood.

As noted above, peptides comprising CTL or HTL epitopes of the invention induce immune responses when presented by HLA molecules and contacted with a CTL or HTL specific for an epitope comprised by the peptide. The manner in which the peptide is contacted with the CTL or HTL is not critical to the invention. For instance, the peptide can be contacted with the CTL or HTL either *in vivo* or *in vitro*. If the contacting occurs *in vivo*, the peptide itself can be administered to the patient, or other vehicles, *e.g.*, DNA vectors encoding one or more peptides, viral vectors encoding the peptide(s), liposomes and the like, can be used, as described herein.

For pharmaceutical compositions, the immunogenic peptides of the invention, or DNA encoding them, are generally administered to an individual already infected with HBV. The peptides or DNA encoding them can be administered individually or as fusions of one or more peptide sequences. Those in the incubation phase or the acute phase of infection can be treated with the immunogenic peptides separately or in conjunction with other treatments, as appropriate.

For therapeutic use, administration should generally begin at the first diagnosis of HBV infection. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. In chronic infection, loading doses followed by boosting doses may be required.

Treatment of an infected individual with the compositions of the invention may hasten resolution of the infection in acutely infected individuals. For those individuals susceptible (or predisposed) to developing chronic infection, the compositions are particularly useful in methods for preventing the evolution from acute to chronic infection. Where susceptible individuals are identified prior to or during infection, the

composition can be targeted to them, thus minimizing the need for administration to a larger population.

The peptide or other compositions used for the treatment or prophylaxis of HBV infection can be used, *e.g.,* in persons who have not manifested symptoms of disease but who act as a disease vector. In this context, it is generally important to provide an amount of the peptide epitope delivered by a mode of administration sufficient to effectively stimulate a cytotoxic T cell response; compositions which stimulate helper T cell responses can also be given in accordance with this embodiment of the invention.

The dosage for an initial immunization (*i.e.,* therapeutic or prophylactic administration) generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg. Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. Boosting dosages of between about 1.0 μg to about 50000 μg of peptide pursuant to a boosting regimen over weeks to months may be administered depending upon the patient's response and condition as determined by measuring the specific activity of CTL and/or HTL obtained from the patient's blood. The peptides and compositions of the present invention may be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, as a result of the minimal amounts of extraneous substances and the relative nontoxic nature of the peptides in preferred compositions of the invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions relative to these stated dosage amounts.

Thus, for treatment of chronic infection, a representative dose is in the range disclosed above, namely where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg, preferably from about 500 μg to about 50,000 μg per 70 kilogram patient. Initial doses followed by boosting doses at established intervals, *e.g.,* from four weeks to six months, may be required, possibly for a prolonged period of time to effectively immunize an individual. In the case of chronic infection, administration should continue until at least clinical symptoms or laboratory tests indicate that the viral infection has been eliminated or substantially abated and for a period thereafter. The dosages, routes of administration, and dose schedules are adjusted in accordance with methodologies known in the art.

The pharmaceutical compositions for therapeutic treatment are intended for parenteral, topical, oral, intrathecal, or local administration. Preferably, the

pharmaceutical compositions are administered parentally, *e.g.*, intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, *e.g.*, water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservatives, and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, *etc.*

The concentration of peptides of the invention in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, *etc.*, in accordance with the particular mode of administration selected.

A human unit dose form of the peptide composition is typically included in a pharmaceutical composition that comprises a human unit dose of an acceptable carrier, preferably an aqueous carrier, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans (*see*, *e.g.*, Remington's Pharmaceutical Sciences, 17th Edition, A. Gennaro, Editor, Mack Publising Co., Easton, Pennsylvania, 1985)

The peptides of the invention may also be administered via liposomes, which serve to target the peptides to a particular tissue, such as lymphoid tissue, or to target selectively to infected cells, as well as to increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations, the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes either filled or decorated with a desired peptide of the invention can be directed to the site of lymphoid cells, where the liposomes then deliver

the peptide compositions. Liposomes for use in accordance with the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, *e.g.*, liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, *e.g.*, Szoka, *et al., Ann. Rev. Biophys. Bioeng.* 9:467 (1980), and U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

For targeting cells of the immune system, a ligand to be incorporated into the liposome can include, *e.g.*, antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide may be administered intravenously, locally, topically, *etc.* in a dose which varies according to, *inter alia*, the manner of administration, the peptide being delivered, and the stage of the disease being treated.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, *e.g.*, lecithin for intranasal delivery.

IV.M. Kits

The peptide and nucleic acid compositions of this invention can be provided in kit form together with instructions for vaccine administration. Typically the kit would include desired peptide compositions in a container, preferably in unit dosage form and instructions for administration. An alternative kit would include a minigene construct with desired nucleic acids of the invention in a container, preferably in unit dosage form together with instruction for administration. Lymphokines such as IL-2 or IL-12 may also be included in the kit. Other kit components that may also be desirable include, for example, a sterile syringe, booster dosages, and other desired excipients.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of non-critical parameters that can be changed or modified to yield alternative embodiments in accordance with the invention.

V. EXAMPLES

Example 1: HLA Class I and Class II Binding Assays

The following example of peptide binding to HLA molecules demonstrates quantification of binding affinities of HLA class I and class II peptides. Binding assays can be performed with peptides that are either motif-bearing or not motif-bearing.

Epstein-Barr virus (EBV)-transformed homozygous cell lines, fibroblasts, CIR, or 721.22 transfectants were used as sources of HLA class I molecules. These cells were maintained *in vitro* by culture in RPMI 1640 medium supplemented with 2mM L-glutamine (GIBCO, Grand Island, NY), 50μM 2-ME, 100μg/ml of streptomycin, 100U/ml of penicillin (Irvine Scientific) and 10% heat-inactivated FCS (Irvine Scientific, Santa Ana, CA). Cells were grown in 225-cm^2 tissue culture flasks or, for large-scale cultures, in roller bottle apparatuses. The specific cell lines routinely used for purification of MHC class I and class II molecules are listed in Table XXIV.

Cell lysates were prepared and HLA molecules purified in accordance with disclosed protocols (Sidney *et al.*, *Current Protocols in Immunology* 18.3.1 (1998); Sidney, *et al., J. Immunol.* 154:247 (1995); Sette, *et al., Mol. Immunol.* 31:813 (1994)). Briefly, cells were lysed at a concentration of 10^8 cells/ml in 50 mM Tris-HCl, pH 8.5,

containing 1% Nonidet P-40 (Fluka Biochemika, Buchs, Switzerland), 150 mM NaCl, 5 mM EDTA, and 2 mM PMSF. Lysates were cleared of debris and nuclei by centrifugation at 15,000 x g for 30min.

HLA molecules were purified from lysates by affinity chromatography. Lysates prepared as above were passed twice through two pre-columns of inactivated Sepharose CL4-B and protein A-Sepharose. Next, the lysate was passed over a column of Sepharose CL-4B beads coupled to an appropriate antibody. The antibodies used for the extraction of HLA from cell lysates are listed in Table XXV. The anti-HLA column was then washed with 10-column volumes of 10mM Tris-HCL, pH 8.0, in 1% NP-40, PBS, 2-column volumes of PBS, and 2-column volumes of PBS containing 0.4% n-octylglucoside. Finally, MHC molecules were eluted with 50mM diethylamine in 0.15M NaCl containing 0.4% n-octylglucoside, pH 11.5. A 1/25 volume of 2.0M Tris, pH 6.8, was added to the eluate to reduce the pH to ~8.0. Eluates were then be concentrated by centrifugation in Centriprep 30 concentrators at 2000 rpm (Amicon, Beverly, MA). Protein content was evaluated by a BCA protein assay (Pierce Chemical Co., Rockford, IL) and confirmed by SDS-PAGE.

A detailed description of the protocol utilized to measure the binding of peptides to Class I and Class II MHC has been published (Sette *et al.*, *Mol. Immunol.* 31:813, 1994; Sidney *et al.*, in *Current Protocols in Immunology*, Margulies, Ed., John Wiley & Sons, New York, Section 18.3, 1998). Briefly, purified MHC molecules (5 to 500nM) were incubated with various unlabeled peptide inhibitors and 1-10nM ^{125}I-radiolabeled probe peptides for 48h in PBS containing 0.05% Nonidet P-40 (NP40) (or 20% w/v digitonin for H-2 IA assays) in the presence of a protease inhibitor cocktail. The final concentrations of protease inhibitors (each from CalBioChem, La Jolla, CA) were 1 mM PMSF, 1.3 nM 1.10 phenanthroline, 73 μM pepstatin A, 8mM EDTA, 6mM N-ethylmaleimide (for Class II assays), and 200 μM N alpha-p-tosyl-L-lysine chloromethyl ketone (TLCK). All assays were performed at pH 7.0 with the exception of DRB1*0301, which was performed at pH 4.5, and DRB1*1601 (DR2w21 ·1) and DRB4*0101 (DRw53), which were performed at pH 5.0. pH was adjusted as described elsewhere (see Sidney *et al.*, in *Current Protocols in Immunology*, Margulies, Ed., John Wiley & Sons, New York, Section 18.3, 1998).

Following incubation, MHC-peptide complexes were separated from free peptide by gel filtration on 7.8 mm x 15 cm TSK200 columns (TosoHaas 16215,

Montgomeryville, PA), eluted at 1.2 mls/min with PBS pH 6.5 containing 0.5% NP40 and 0.1% NaN_3. Because the large size of the radiolabeled peptide used for the DRB1*1501 (DR2w2·1) assay makes separation of bound from unbound peaks more difficult under these conditions, all DRB1*1501 (DR2w2·1) assays were performed using a 7.8mm x 30cm TSK2000 column eluted at 0.6 mls/min. The eluate from the TSK columns was passed through a Beckman 170 radioisotope detector, and radioactivity was plotted and integrated using a Hewlett-Packard 3396A integrator, and the fraction of peptide bound was determined.

Radiolabeled peptides were iodinated using the chloramine-T method. The specific radiolabeled probe peptide utilized in each assay, and its assay specific IC_{50} nM, is summarized in Table XXIV. Typically, in preliminary experiments, each MHC preparation was titered in the presence of fixed amounts of radiolabeled peptides to determine the concentration of HLA molecules necessary to bind 10-20% of the total radioactivity. All subsequent inhibition and direct binding assays were performed using these HLA concentrations.

Since under these conditions [label]<[HLA] and IC50≥[HLA], the measured IC_{50} values are reasonable approximations of the true K_D values. Peptide inhibitors are typically tested at concentrations ranging from 120 μg/ml to 1.2 ng/ml, and are tested in two to four completely independent experiments. To allow comparison of the data obtained in different experiments, a relative binding figure is calculated for each peptide by dividing the IC_{50} of a positive control for inhibition by the IC_{50} for each tested peptide (typically unlabeled versions of the radiolabeled probe peptide). For database purposes, and inter-experiment comparisons, relative binding values are compiled. These values can subsequently be converted back into IC_{50} nM values by dividing the IC_{50} nM of the positive controls for inhibition by the relative binding of the peptide of interest. This method of data compilation has proven to be the most accurate and consistent for comparing peptides that have been tested on different days, or with different lots of purified MHC.

Because the antibody used for HLA-DR purification (LB3.1) is α-chain specific, β_1 molecules are not separated from·β_3 (and/or β_4 and β_5) molecules. The β_1 specificity of the binding assay is obvious in the cases of DRB1*0101 (DR1), DRB1*0802 (DR8w2), and DRB1*0803 (DR8w3), where no β_3 is expressed. It has also been demonstrated for DRB1*0301 (DR3) and DRB3*0101 (DR52a), DRB1*0401 (DR4w4), DRB1*0404

(DR4w14), DRB1*0405 (DR4w15), DRB1*1101 (DR5), DRB1*1201 (DR5w12), DRB1*1302 (DR6w19) and DRB1*0701 (DR7). The problem of β chain specificity for DRB1*1501 (DR2w2·1), DRB5*0101 (DR2w2·2), DRB1*1601 (DR2w21·1), DRB5*0201 (DR2w21·3), and DRB4*0101 (DRw53) assays is circumvented by the use of fibroblasts. Development and validation of assays with regard to DRβ molecule specificity have been described previously (see, *e.g.*, Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998).

Binding assays as outlined above may be used to analyze supermotif and/or motif-bearing epitopes as, for example, described in Example 2.

Example 2. Identification of Conserved HLA Supermotif CTL Candidate Epitopes

Vaccine compositions of the invention may include multiple epitopes that comprise multiple HLA supermotifs or motifs to achieve broad population coverage. This example illustrates the identification of supermotif-bearing epitopes for the inclusion in such a vaccine composition. Calculation of population coverage was performed using the strategy described below. Epitopes were then selected to bear an HLA-A2, -A3, or -B7 supermotif or an HLA-A1 or -A24 motif.

Computer searches and algorthims for identification of supermotif and/or motif-bearing epitopes

Computer searches for epitopes bearing HLA Class I or Class II supermotifs or motifs were performed as follows. All translated HBV isolate sequences were analyzed using a text string search software program, *e.g.*, MotifSearch 1.4 (D. Brown, San Diego) to identify potential peptide sequences containing appropriate HLA binding motifs; alternative programs are readily produced in accordance with information in the art in view of the motif/supermotif disclosure herein. Furthermore, such calculations can be made mentally. Identified A2-, A3-, and DR-supermotif sequences were scored using polynomial algorithms to predict their capacity to bind to specific HLA-Class I or Class II molecules. These polynomial algorithms take into account both extended and refined motifs (that is, to account for the impact of different amino acids at different positions), and are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA molecule interactions can be approximated as a linear polynomial function of the type:

$$\text{“}\Delta G\text{”} = a_{1i} \times a_{2i} \times a_{3i} \ldots\ldots \times a_{ni}$$

where a_{ji} is a coefficient which represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. The crucial assumption of this method is that the effects at each position are essentially independent of each other (i.e., independent binding of individual side-chains). When residue j occurs at position i in the peptide, it is assumed to contribute a constant amount j_i to the free energy of binding of the peptide irrespective of the sequence of the rest of the peptide. This assumption is justified by studies from our laboratories that demonstrated that peptides are bound to MHC and recognized by T cells in essentially an extended conformation (data omitted herein).

The method of derivation of specific algorithm coefficients has been described in Gulukota *et al.*, *J. Mol. Biol.* 267:1258-126, 1997; (see also Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; and Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). Briefly, for all i positions, anchor and non-anchor alike, the geometric mean of the average relative binding (ARB) of all peptides carrying j is calculated relative to the remainder of the group, and used as the estimate of j_i. For Class II peptides, if multiple alignments are possible, only the highest scoring alignment is utilized, following an iterative procedure. To calculate an algorithm score of a given peptide in a test set, the ARB values corresponding to the sequence of the peptide are multiplied. If this product exceeds a chosen threshold, the peptide is predicted to bind. Appropriate thresholds are chosen as a function of the degree of stringency of prediction desired.

Selection of HLA-A2 supertype cross-reactive peptides

Complete sequences from 20 HBV isolates were aligned, then scanned, utilizing a customized computer program, to identify conserved 9- and 10-mer sequences containing the HLA-A2-supertype main anchor specificity.

A total of 150 conserved and motif-positive sequences were identified. These peptides were then evaluated for the presence of A*0201 preferred secondary anchor residues using an A*0201-specific polynomial algorithm. A total of 85 conserved, motif-positive sequences were selected and synthesized.

These 85 conserved, motif-containing 9- and 10-mer peptides were then tested for their capacity to bind purified HLA-A*0201 molecules in vitro. Thirty-four peptides were found to be good A*0201 binders ($IC_{50} \leq 500$ nM); 15 were high binders ($IC_{50} \leq 50$ nM) and 19 were intermediate binders (IC_{50} of 50-500 nM) (Table XXVI).

In the course of independent analyses, 25 conserved, HBV-derived, 8 or 11-mer sequences with appropriate A2-supertype main anchors were also synthesized and tested for A*0201 binding. One peptide, HBV env 259 11-mer (peptide 1147.14), bound A*0201 with an IC_{50} of 500 nM, or less, and has been included in Table XXVI. Also shown in Table XXVI is an analog peptide, representing a single substitution of the HBV pol 538 9-mer peptide, which binds A*0201 with an IC_{50} of 5.1 nM (see below).

Thirty of the 36 A*0201 binders were subsequently tested for the capacity to bind to additional A2-supertype alleles (A*0202, A*0203, A*0206, and A*6802). As shown in Table XXVI, 15/30 (50%) peptides were found to be A2-supertype cross-reactive binders, binding at least 3 of the 5 A2-supertype alleles tested. These 15 peptides were selected for further analysis.

Selection of HLA-A3 supermotif-bearing epitopes

The sequences from the same 20 isolates were also examined for the presence of conserved peptides with the HLA-A3-supermotif primary anchors. A total of 80 conserved 9- or 10-mer motif-containing sequences were identified. Further analysis using the A03 and A11 algorithms identified 40 sequences which scored high in either or both algorithms. Thirty-six of the corresponding peptides were synthesized and tested for binding to HLA-A*03 and HLA-A*11, the two most prevalent A3-supertype alleles. Twenty-three peptides were identified which bound A3 and/or A11 with affinities or IC_{50} values of $\leq$ 500 nM (Table XXVII).

In the course of an independent series of studies 30 HBV-derived 8-mer, and 24 11-mer sequences, conserved in 75% or more of the isolates, were selected and tested for A*03 and A*11 binding. Four 8-mers and 9 11-mers were found to bind either or both alleles (Table XXVII). Finally, four 9-mer, and one 10-mer, conserved HBV-derived peptides not selected using the search criteria outlined above, but which have been shown to bind A*03 and/or A*11, have been identified, and are included in Table XXVII. Two of these peptides contain non-canonical anchors (peptides 20.0131, and 20.0130), and the other 3 are algorithm negative (peptides 1142.05, 1099.03, and 1090.15).

Thirty-eight of the 41 peptides binding A*03 and/or A*11 were subsequently tested for binding crossreactivity to the other common A3-supertype alleles (A*3101, A*3301, and A*6801). It was found that 17 of these peptides were A3-supertype cross-reactive, binding at least 3 of the 5 A3-supertype alleles tested (Table XXVII).

Selection of HLA-B7 supermotif bearing epitopes

When the same 20 isolates were also analyzed for the presence of conserved 9- or 10-mer peptides with the HLA-B7-supermotif, 46 sequences were identified. Thirty-four of the corresponding peptides were synthesized and tested for binding to HLA-B*0702, the most common B7-supertype allele. Nine peptides bound B*0702 with an IC_{50} value of ≤ 500 nM (Table XXVIII). These 9 peptides were then tested for binding to other common B7-supertype alleles (B*3501, B*51, B*5301, and B*5401). Five of the 9 B*0702 binders were capable of binding to 3 or more of the 5 B7-supertype alleles tested.

In separate studies investigating the secondary anchor requirements of B7-supertype alleles, all available peptides with the B7-supermotif were tested for binding to all B7 supertype alleles. As a result, all 34 peptides described above were also tested for binding to other B7-supertype alleles. These experiments identified an additional 10 peptides which bound at least one B7-supertype allele with an IC_{50} value ≤ 500 nM, including 2 peptides which bound 3 or more alleles. These 10 peptides are also shown in Table XXVIII.

Because of the low numbers of conserved B7-supertype degenerate HBV-derived 9- and 10-mer peptides, compared to the A2- and A3-supertypes, the 20 isolates were again examined to identify conserved, motif-containing 8- and 11-mers. This re-scan identified 51 peptides. Thirty-one of these were synthesized and tested for binding to each of the 5 most common B7-supertype alleles. Nineteen 8- and 11-mer peptides bound with high or intermediate affinity to at least one B7-supertype allele (IC_{50} ≤ 500 nM) (Table XXVIII). Two peptides were degenerate binders, binding 3 of the 5 alleles tested.

In summary, a total of 9 HBV-derived peptides, conserved in 75% or more of the isolates analyzed, have been identified which are degenerate B7-supertype binders (Table XXVIII).

Selection of A1 and A24 motif-bearing epitopes

To further increase population coverage, HLA-A1 and -A24 epitopes have been incorporated into the present analysis. A1 is, on average, present in 12%, and A24 is present in approximately 29%, of the population across five different major ethnic groups (Caucasian, North American Black, Chinese, Japanese, and Hispanic). Combined, these

alleles would be represented with an average frequency of 39% in these same populations. The total coverage across the major ethnicities when A1 and A24 are combined with the coverage of the A2-, A3- and B7-supertype alleles is >95.4%; by comparison, coverage by combing the A2-, A3-, and B7-supertypes is 86.2%.

Systematic analyses of HBV for A1 and A24 binders have yet to be completed . However, in the course of independent studies, 15 conserved HBV-derived peptides have been identified that bind A*0101 with IC50 less than 500 nM (Table XXIX); 7 of these bind with IC_{50} less than 100 nM . In a similar context, 14 conserved A*2402 binding HBV-derived peptides have also been identified, 6 of which bind A*2402 with IC_{50} less than 100 nM (Table XXIX).

Example 3: Confirmation of Immunogenicity

*Evaluation of A*0201 immunogenicity*

The immunogenicity analysis of the 15 HBV-derived HLA-A2 supertype cross-reactive peptides identified above is summarized in Table XXX. Peptides were screened for immunogenicity in at least one of three systems. Peptides were screened for the induction of primary antigen-specific CTL *in vitro* using human PBMC (Wentworth *et al.*, *Molec. Immunol.* 32:603, 1995); this data is indicated as "primary CTL" in Table XXX.

The protocol for *in vitro* induction of primary antigen-specific CTL from human PBMC has been described by Wentworth et al (Wentworth *et al.*, *Molec. Immunol.* 32:603, 1995). PBMC from normal donors which had been enriched for CD8+ T cells were incubated with peptide loaded antigen-presenting cells (SAC-I activated PBMCs) in the presence of IL-7. After seven days cultures were restimulated using irradiated autologous adherent cells pulsed with peptide and then tested for cytotoxic activity seven days later.

In addition, HLA transgenic mice were used to evaluate peptide immunogenicity; this data is indicated as "transgenic CTL" in Table XXX. Previous studies have shown that CTL induced in A*0201/Kb transgenic mice exhibit specificity similar to CTL induced in humans (Vitiello *et al.*, *J. Exp. Med.* 173:1007, 1991; Wentworth *et al.*, *Eur. J. Immunol.* 26:97, 1996).

CTL induction in transgenic mice following peptide immunization has been described by Vitiello *et al.* (Vitiello *et al.*, *J. Exp. Med.* 173:1007, 1991) and Alexander *et al.* (Alexander *et al.*, *J. Immunol.* 159:4753, 1997). Briefly, synthetic peptides (50

μg/mouse) and the helper epitope HBV core 128 (140 μg/mouse) were emulsified in incomplete Freund's adjuvant (IFA) and injected subcutaneously at the base of the tail. Eleven days after injection, splenocytes were incubated in the presence of peptide-loaded syngenic LPS blasts. After six days cultures were assayed for cytotoxic activity using peptide-pulsed targets.

Peptides were also tested for the ability to stimulate recall CTL responses in acutely infected HBV patients (Bertoni *et al., J. Clin. Invest.* 100:503, 1997; Rehermann *et al., J. Clin. Invest.* 97:1655-1665, 1996; Nayersina *et al., J. Immunol.* 150:4659, 1993); these data are indicated as "patient CTL" in Table XXX. Patient immunogenicity data is particularly informative as it indicates that a peptide is recognized during the course of a natural infection. These data demonstrate that a peptide is processed and presented in human cells that would represent the targets for CTL. Moreover, this data is especially relevant for vaccine design as the induction of CTL responses in patients has been correlated to the resolution of infection.

For the evaluation of recall CTL responses, screening was carried out as described by Bertoni *et al.* (Bertoni *et al., J. Clin. Invest.* 100:503, 1997). Briefly, PBMC from patients acutely infected with HBV were cultured in the presence of 10μg/ml of synthetic peptide. After seven days, the cultures were restimulated with peptide. The cultures were assayed for cytotoxic activity on day 14 using target cells pulsed with peptide.

Of the 15 A2 supertype binding peptides, 11 were found to be immunogenic in at least one of the systems utilized. Five of the 11 peptides had previously been identified in the patients with acute HBV (Bertoni *et al., J. Clin. Invest.* 100:503, 1997). Five additional degenerate peptides (1069.06, 1090.77, 1147.14, 927.42 and 927.46) induced CTL responses in HLA-A*0201 transgenic mice. The 11 immunogenic supertype cross-reactive peptides are encoded by three HBV antigens; core, envelope and polymerase.

This set of 11 immunogenic A2-supermotif-bearing epitopes includes one analog peptide, 1090.77. The wild type peptide, 1090.14, from which this analog is derived is A2-supertype non-cross-reactive, but has been shown to be recognized in recall CTL responses from acute HBV patients, and to be immunogenic in HLA-A*0201 transgenic mice as well as primary human cultures (Table XXX). Further studies addressing the cross recognition of the wild type peptide 1090.14 and the 1090.77 analog are described in detail below.

In the course of independent analyses, 14 of the non-cross-reactive peptides shown in Table XXXb, including 1090.14, were found to be immunogenic in at least one

system. Five peptides of these peptides were recognized in patients; 4 peptides induced CTL in transgenic mice.

In conclusion, 11 A2-supertype cross-reactive peptides have been identified that are capable of exhibiting immunogenicity in at least one of the three systems examined.

*Evaluation of A*03/A11 immunogenicity*

Seven of the 17 A3-supertype cross-reactive peptides have been evaluated for immunogenicity (Table XXXI). As described in the previous section, A3-supermotif-bearing peptides were screened using primary cultures, patient responses, or HLA-A11 transgenic mice (Alexander *et al.*, *J. Immunol.* 159:4753, 1997). With the exception of peptide 1.0219, all of the conserved cross-reactive peptides listed in Table insert table XXXI were found to be immunogenic.

Additionally, a poorly conserved peptide (1150.51; 40% conserved) which exhibits cross-reactive supertype binding was found to be immunogenic in transgenic mice, and has been included in Table XXXI. Two other conserved, but non-cross-reactive, peptides have also been shown to be recognized in acutely infected HBV patients (Bertoni *et al.*, *J. Clin. Invest.* 100:503, 1997). These epitiopes are shown in Table XXXI.

It is notable that for 7 of the 8 conserved immunogenic HBV-derived A3-supermotif-bearing epitopes, including all 6 of the cross-reactive peptides, positive data was obtained in patients. These epitopes are predominantly derived from the polymerase protein sequence, with only one epitope being derived from the core protein sequence. While a number of cross-reactive peptides have been identified in the X antigen (Table XXXI), to date these peptides have not been screened for immunogenicity.

In summary, 7 A3-supermotif-bearing, cross-reactive peptides have been identified that are recognized by CTL in acutely infected patients, or induce CTL in HLA-transgenic mice.

Evaluation of B7 immunogenicity

The immunogenicity studies involving the HBV-derived HLA-B7-supermotif-bearing, cross-reactive peptides is summarized in Table XXXII. HLA-B7 peptides were screened exclusively in human systems measuring responses in either primary cultures or acutely infected HBV patients. Of the 7 degenerate peptides screened, 4 were shown to be immunogenic. One non-crossreactive peptide (XRN<3), 1147.04, was also shown to

be recognized in acutely infected HBV patients (Bertoni *et al.*, *J. Clin. Invest.* 100:503, 1997; see TableXXXII).

In summary, 5 conserved HBV-derived B7-supermotif-bearing epitopes that are recognized in acutely infected HBV patients have been identified. These epitopes afford coverage of 4 different HBV antigens (core, envelope, polymerase and X).

Example 4: Implementation of the Extended Supermotif to Improve the Binding Capacity of Native Peptides by Creating Analogs

HLA motifs and supermotifs (comprising primary and/or secondary residues) are useful in preparing highly cross-reactive native peptides, as demonstrated herein. Moreover, the definition of HLA motifs and supermotifs also allows one to engineer highly cross-reactive epitopes by identifying residues within a native peptide sequence which can be analoged, or "fixed", to confer upon a peptide certain characteristics, *e.g.*, greater cross-reactivity within the group of HLA molecules that make-up the supertype, and/or greater binding affinity for some or all of those HLA molecules Examples of analog peptides that exhibit modulated binding affinity are provided.

Analoging at Primary Anchor Residues

It has been shown that class I peptide ligands can be modified, or "fixed" to increase their binding affinity and/or degeneracy (Sidney *et al.*, *J. Immunol.* 157:3480, 1996). These fixed peptides may also demonstrate increased immunogenicity and crossreactive recognition by T cells specific for the wild type epitope (Parkhurst *et al.*, *J. Immunol.* 157:2539, 1996; Pogue *et al.*, *Proc. Natl. Acad. Sci. USA* 92:8166, 1995). Specifically, the main anchors of A2 supertype peptides may be"fixed", or analoged, to L or V (or M, if natural) at position 2, and V at the C-terminus. As indicated in Table XXVI, 9 of the 14 A2-supertype cross-reactive binding peptides are "fixable" by these criteria, as are 16 of the 21 non-cross-reactive binders. Ideal candidates for fixing would be peptides which bind at least 3 A2-supertype allele-specific molecules with $IC_{50} \leq 5000$ nM.

An example of the efficacy of this strategy to generate more broadly cross-reactive epitopes is provided by the case of peptide 1090.14 (Table XXVI). Previously, this peptide was shown to be highly immunogenic in each of the systems examined. However, it only exhibits binding to a single A2-supertype allele-specific molecule,

A*0201. The non-crossreactive binding capacity of this epitope limits the population coverage and consequently the value of including this peptide in a candidate vaccine. In an effort to increase binding affinity and cross-reactivity the C-terminus of peptide 1090.14 was altered from 'alanine' to the A2-supermotif preferred residue 'valine'. This change resulted in a dramatic (40-fold) increase in binding capacity for A*0201 (from 200 nM to 5.1 nM), but also produced a peptide capable of binding 3 other A2-supertype allele-specific molecules. (see peptide 1090.77, Table XXVI).

Studies with HLA-A*0201 transgenic mice have shown that the CTL response from mice immunized with the 1090.77 peptide recognize target cells loaded with either the naturally occurring peptide 1090.14 or the valine-substituted analog (*i.e.*, 1090.77). In fact, the lysis effected by 1090.77 induced CTL was indistinguishable regardless whether the analog or the wild-type sequence was used to load the target cells (B. Livingston, unpublished data).

The relevance of these observations for the design of vaccine constructs is indicated by studies in which chronic HBV patients were treated with the potent viral replication inhibitor, lamivudine. Extended therapy with lamivudine resulted in the selection of drug-resistant strains of HBV that have a substitution of valine for methione at position 2 in the 1090.14 epitope, suggesting that epitope-based vaccines used in combination with lamivudine may need to have the ability to induce CTL responses that recognize both wild type and mutant sequences.

To demonstrate that cross-recognition is possible between the native peptide (1090.14), the analog peptide, and the lamivudine induced mutant M2 peptide, CTL were generated using the 1090.77 analog peptide. These CTL cultures were then stimulated with either the wild type peptide (1090.14), or the lamivudine induced mutant M2 peptide. The ability of these CTL to then lyse target cells loaded with either the wild type, or the lamivudine induced mutant peptide was then assayed. Target cells presenting either peptide were similarly lysed by either CTL culture (Table XXVI).

These studies demonstrate how analoging a peptide can result in dramatically increased HLA-A2 supertype degeneracy while still allowing cross-recognition between wildtype and mutant epitopes. More specifically, these results indicate that a vaccine utilizing the analog peptide 1090.77 should stimulate a response that will recognize both wild-type and lamivudine-resistant strains of HBV.

Similarly, analogs of HLA-A3 supermotif-bearing epitopes may also be generated. For example, peptides could be analogued to possess a preferred V at position

2, and R or K at the C-terminus. Twelve of the A3-supertype degenerate peptides identified in Table XXVII are candidates for main anchor fixing, as are 19 of the 24 non-cross-reactive binders.

Analog peptides are initially tested for binding to A*03 and A*11, and those that demonstrate equivalent, or improved, binding capacity relative to the parent peptide would then be tested for A3-supertype cross-reactivity. Analogs demonstrating improved cross-reactivity are then further evaluated for immunogenicity, as necessary.

Typically, it is more difficult to identify B7 supermotif-bearing epitopes. As in the cases of A2- and A3-supertype epitopes, a peptide analoguing strategy can be utilized to generate additional B7 supermotif-bearing epitopes with increased cross-reactive binding. In general, B7 supermotif-bearing peptides should be fixed to possess P in position 2, and I at their C-terminus.

Analogs representing primary anchor single amino acid residues substituted with I residues at the C-terminus of two different B7-like peptides (HBV env 313 and HBV pol 541) were synthesized and tested for their B7-supertype binding capacity. It was found that the I substitution had an overall positive effect on binding affinity and/or cross-reactivity in both cases. In the case of HBV env 313 the I9 (I at C-terminal position 9) replacement was effective in increasing cross-reactivity from 4 to 5 alleles bound by virtue of an almost 400-fold increase B*5401 binding affinity. In the case of HBV pol 541, increased cross-reactivity was similarly achieved by a substantial increase in B*5401 binding. Also, significant gains in binding affinity for B*0702, B51, and B*5301 were observed with the HBV pol 541 I9 analog.

Analoging at Secondary Anchor Residues

Moreover, HLA supermotifs are of value in engineering highly cross-reactive peptides by identifying particular residues at secondary anchor positions that are associated with such cross-reactive properties. Demonstrating this, the capacity of a second set of peptides representing discreet single amino acid substitutions at positions one and three of five different B7-supertype binding peptides were synthesized and tested for their B-7 supertype binding capacity. In 4/4 cases the effect of replacing the native residue at position 1 with the aromatic residue F (an "F1" substitution) resulted in an increase in cross-reactivity, compared to the parent peptide, and, in most instances, binding affinity was increased three-fold or better (Table XXVIII). More specifically, for HBV env 313, MAGE2 170, and HBV core 168 complete supertype cross-reactivity was

achieved with the F1 substitution analogs. These gains were achieved by dramatically increasing B*5401 binding affinity. Also, gains in affinity were noted for other alleles in the cases of HBV core 168 (B*3501 and B*5301) and MAGE2 170 (B*3501, B51 and B*5301). Finally, in the case of MAGE3 196, the F1 replacement was effective in increasing cross-reactivity because of gains in B*0702 binding. An almost 70-fold increase in B51 binding capacity was also noted.

Two analogs were also made using the supermotif positive F substitution at position three (an "F3" substitution). In both instances increases in binding affinity and cross-reactivity were achieved. Specifically, in the case of HBV pol 541, the F3 substitution was effective in increasing cross-reactivity by virtue of its effect on B*5401 binding. In the case of MAGE3 196, complete supertype cross-reactivity was achieved by increasing B*0702 and B*3501 binding capacity. Also, in the case of MAGE3 196, it is notable that increases in binding capacity between 40- and 5000-fold were obtained for B*3501, B51, B*5301, and B*5401.

In conclusion, these data demonstrate that by the use of even single amino acid substitutions, it is possible to increase the binding affinity and/or cross-reactivity of peptide ligands for HLA supertype molecules.

Example 5: Identification of conserved HBV-derived sequences with HLA-DR binding motifs

Peptide epitopes bearing an HLA class II supermotif or motif may also be identified as outlined below using methodology similar to that described in Examples 1-3.

Selection of HLA-DR-supermotif-bearing epitopes

HLA-Class II molecules bind peptides typically between 12 and 20 residues in length. However, similar to HLA-Class I, the specificity and energy of interaction is usually contained within a short core region of about 9 residues. Most DR molecules share an overlapping specificity within this 9-mer core in which a hydrophobic residue in position 1 (P1) is the main anchor (O'Sullivan *et al.*, *J. Immunol.* 147:2663, 1991; Southwood *et al.*, *J. Immunol.* 160:3363, 1998). The presence of small or hydrophobic residues in position 6 (P6) is also important for most DR-peptide interactions. This overlapping P1-P6 specificity, within a 9-mer core region, has been defined as the DR-supermotif. Unlike Class I molecules, DR molecules are open at both ends of the binding groove, and can therefore accommodate longer peptides of varying length. Indeed, while

most of the energy of peptide-DR interactions appears to be contributed by the core region, flanking residues appear to be important for high affinity interactions. Also, although not strictly necessary for MHC binding, flanking residues are clearly necessary in most instances for T cell recognition.

To identify HBV-derived DR cross-reactive HTL epitopes, the same 20 HBV polyproteins that were scanned for the identification of HLA Class I motif sequences were scanned for the presence of sequences with motifs for binding HLA-DR. Specifically, 15-mer sequences comprised of a DR-supermotif containing 9-mer core, and three residue N- and C-terminal flanking regions, were selected. It was also required that 100% of the 15-mer sequence be conserved in at least 85% (17/20) of the HBV strains scanned. Using these criteria, 36 non-redundant sequences were identified. Thirty-five of these peptides were subsequently synthesized.

Algorithms for predicting peptide binding to DR molecules have also been developed (Southwood *et al.*, *J. Immunol.* 160:3363, 1998). These algorithms, specific for individual DR molecules, allow the scoring and ranking of 9-mer core regions. Using selection tables, it has been found that these algorithms efficiently select peptide sequences with a high probability of binding the appropriate DR molecule. Additionally, it has been found that running algorithms, specifically those for DR1, DR4w4, and DR7, sequentially can efficiently select DR cross-reactive peptides.

To see if these algorithms would identify additional peptides, the same HBV polyproteins used above were re-scanned for the presence of 15-mer peptides where 100% of the 9-mer core region was ^{3}85% (17/20 strains) conserved. Next, the 9-mer core region of each of these peptides was scored using the DR1, DR4w4, and DR7 algorithms. As a result, 8 additional sequences were identified and synthesized.

In summary, 44 15-mer peptides in which a 9-mer core region contained the DRsupermotif, or was selected using an algorithm predicting DR-binding sequences, were identified. Forty-three of these peptides were synthesized (Table XXXIII).

While performing the analyses of HBV-derived peptides described above, 9 peptides predicted on the basis of their DR1, DR4w4, and DR7 algorithm profiles to be DR-cross-reactive binding peptides, but which have 9-mer core regions that are only 80% conserved, were also identified. An additional peptide which contains a DR-supermotif core region that is 95% conserved, but is located only one residue removed from the N-terminus, was previously synthesized. These 10 peptides were also selected for further analysis, and are shown in Table XXXIII.

Finally, 2 peptides, CF-08 and 1186.25, which are redundant with a peptide selected above (27.0280), were considered for additional analysis. Peptide 1186.25 contains multiple DR-supermotif sequences. Peptide CF-08 is a 20-mer that nests both 27.0280 and 1186.25. These peptides are shown in Table XXXIII.

The 55 HBV-derived peptides identified above were tested for their capacity to bind common HLA-DR alleles. To maximize both population coverage, and the relationships between the binding repertoires of most DR alleles (see, *e.g.*, Southwood *et al.*, *J. Immunol.* 160:3363, 1998), peptides were screened for binding to sequential panels of DR assays. The composition of these screening panels, and the phenotypic frequency of associated antigens, are shown in Table XXXIV. All peptides were initially tested for binding to the alleles in the primary panel: DR1, DR4w4, and DR7. Only peptides binding at least 2 of these 3 alleles were then tested for binding in the secondary assays (DR2w2 ß1, DR2w2 ß2, DR6w19, and DR9). Finally, only peptides binding at least 2 of the 4 secondary panel alleles, and thus 4 of 7 alleles total, were screened for binding in the tertiary assays (DR4w15, DR5w11, and DR8w2).

Upon testing, it was found that 25 of the original 55 peptides (45%) bound two or more of the primary panel alleles. When these 25 peptides were subsequently tested in the secondary assays, 20 were found to bind at least 4 of the 7 DR alleles in the primary and secondary assay panels. Finally, 18 of the 20 peptides passing the secondary screening phase were tested for binding in the tertiary assays. As a result, 12 peptides were shown to bind at least 7 of 10 common HLA-DR alleles. The sequences of these 12 peptides, and their binding capacity for each assay in the primary through tertiary panels, are shown in Table XXXV. Also shown are peptides CF-08 and 857.02, which bound 5/5 and 5/6 of the alleles tested to date, respectively.

In summary, 14 peptides, derived from 12 independent regions of the HBV genome, have been identified that are capable of binding multiple HLA-DR alleles. This set of peptides includes at least 2 epitopes each from the Core (Nuc), Pol, and Env antigens.

Selection of conserved DR3 motif peptides

Because HLA-DR3 is an allele that is prevalent in Caucasian, Black, and Hispanic populations, DR3 binding capacity is an important criterion in the selection of HTL epitopes. However, data generated previously indicated that DR3 only rarely cross-reacts with other DR alleles (Sidney *et al.*, *J. Immunol.* 149:2634-2640, 1992; Geluk *et al.*, *J.*

Immunol. 152:5742-5748, 1994; Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). This is not entirely surprising in that the DR3 peptide-binding motif appears to be distinct from the specificity of most other DR alleles.

To efficiently identify peptides that bind DR3, target proteins were analyzed for conserved sequences carrying one of the two DR3 specific binding motifs reported by Geluk *et al.* (*J. Immunol.* 152:5742-5748, 1994). Eighteen sequences were identified. Eight of these sequences were largely redundant with peptides shown in Table XXXVI, and 3 with peptides that had previously been synthesized for other studies. The 7 unique sequences were synthesized.

Seventeen of the eighteen peptides containing a DR3 motif have been tested for their DR3 binding capacity. Four peptides were found to bind DR3 with an affinity of 1000 nM or better (Table XXXVI).

Example 6. Calculation of phenotypic frequencies of HLA-supertypes in various ethnic backgrounds to determine breadth of population coverage

This example illustrates the assessment of the breadth of population coverage of a vaccine composition comprised of multiple epitopes comprising multiple supermotifs and/or motifs.

In order to analyze population coverage, gene frequencies of HLA alleles were determined. Gene frequencies for each HLA allele were calculated from antigen or allele frequencies utilizing the binomial distribution formulae gf=1-(SQRT(1-af)) (see, *e.g.*, Sidney *et al.*, *Human Immunol.* 45:79-93, 1996). To obtain overall phenotypic frequencies, cumulative gene frequencies were calculated, and the cumulative antigen frequencies derived by the use of the inverse formula [af=1-(1-Cgf)^2].

Where frequency data was not available at the level of DNA typing, correspondence to the serologically defined antigen frequencies was assumed. To obtain total potential supertype population coverage no linkage disequilibrium was assumed, and only alleles confirmed to belong to each of the supertypes were included (minimal estimates). Estimates of total potential coverage achieved by inter-loci combinations were made by adding to the A coverage the proportion of the non-A covered population that could be expected to be covered by the B alleles considered (*e.g.*, total=A+B*(1-A)). Confirmed members of the A3-like supertype are A3, A11, A31, A*3301, and A*6801. Although the A3-like supertype may potentially include A34, A66, and A*7401, these alleles were not included in overall frequency calculations. Likewise, confirmed

members of the A2-like supertype family are A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*6802, and A*6901. Finally, the B7-like supertype-confirmed alleles are: B7, B*3501-03, B51, B*5301, B*5401, B*5501-2, B*5601, B*6701, and B*7801 (potentially also B*1401, B*3504-06, B*4201, and B*5602). Population coverage achieved by combining the A2-, A3- and B7-supertypes is approximately 86% in five major ethnic groups (see Table XXI). Coverage may be extended by including peptides bearing the A1 and A24 motifs. On average, A1 is present in 12% and A24 in 29% of the population across five different major ethnic groups (Caucasian, North American Black, Chinese, Japanese, and Hispanic). Together, these alleles are represented with an average frequency of 39% in these same ethnic populations. The total coverage across the major ethnicities when A1 and A24 are combined with the coverage of the A2-, A3- and B7-supertype alleles is >95%.

Population coverage for HLA class II molecules can be developed analagously based on the present disclosure.

Summary of candidate HLA class I and class II epitopes

In summary, on the basis of the data presented above, 34 conserved CTL epitopes were selected as vaccine candidates (Table XXXVII). Of these 34 epitopes, 7 are derived from core, 18 from polymerase, and 9 from envelope. No epitopes from the X antigen were included in the package as this protein is expressed in low amounts and is, therefore, of less immunological interest.

The population coverage afforded by this panel of CTL epitopes is estimated to exceed 95% in each of 5 major ethnic populations. Using a Monte Carlo analysis (Figure 1), it is predicted that approximately 90% of the individuals in a population comprised of Caucasians, North American Blacks, Japanese, Chinese and Hispanics would recognize five or more of the vaccine candidate epitopes.

While preferred CTL epitopes includes 34 discrete peptides, two peptides are entirely nested within longer peptides, thus effectively reducing the numbers of peptides that would have to be included in a vaccine candidate. Specifically, the A2-restricted peptide 927.15 is nested within the B7-restricted peptide 26.0570 and the B7-restricted peptide 988.05 is nested within the A2-restricted peptide 924.07. Similarly, the A24-restricted peptide 20.0136 and the A2-restricted peptide 1013.01 contain the same core region, differing only at the first amino acid. On a related note, the A2-restricted peptide

1090.14 and the B7-restricted peptide 1147.05 overlap by two amino acids, raising the possibility of delivering these two epitopes as one contiguous peptide sequence.

The set of recommended vaccine candidates includes 9 A2-restricted CTL epitopes; four polymerase-derived epitopes, four envelope-derived epitopes and a core epitope. Seven of these 9 peptides are recognized in recall CTL assays from acute patients. Of the 7 peptides recognized in patients, 2 are non-crossreactive binding peptides. The inclusion of these peptides as potential vaccine candidates stems from the observation that HLA-A*0201 is the predominantly expressed A2-supertype allele in all ethnicities examined. As such, inclusion of non-crossreactive A*0201 binding peptides increases the redundancy of antigen coverage and population coverage. The only two A2-restricted peptides that lack patient immunogenicity data are peptides 1090.77 and 1069.06. The 1090.77 peptide is an analog of a highly immunogenic peptide recognized in acute HBV patients. Although recall responses in patients have not been tested for the ability to recognize the analog peptide, immunogenicity studies conducted in HLA transgenic mice have shown that CTL induced with 1090.77 are capable of recognizing target cells loaded with the naturally occurring sequence. This data indicates that CTL raised to the 1090.77 peptide are cross-reactive and should recognize HBV-infected cells. The 1069.06 peptide was included as a potential vaccine epitope because its high binding affinity for A*6802 results in a greater population coverage. While peptide 1069.06 has not been tested for recognition by acute HBV patients, the peptide is immunogenic in HLA-A2 transgenic mice and primary human cultures.

Preferred CTL epitopes include 7 A3-supertype-resticted peptides; 6 derived from the polymerase antigen, and one from the core region. All of the A3-supertype vaccine candidate peptides are immunogenic in patients. Although peptide 1142.05 is a non-crossreactive A3-restricted peptide, it has been included because it has been shown to be recognized in patients and is capable of binding HLA-A1.

Nine B7-restricted peptides are preferred CTL epitopes. Of this group, 3 epitopes have been shown to be recognized in patients. While one of these peptides, 1147.04, is a non-crossreactive binder, it binds 2 of the major B7 supertype alleles with an IC_{50} or binding affinity value of less than 100 nM. Six B7-supertype epitopes were included as preferred epitopes based on supertype binding. Immunogenicity studies in humans (Bertoni et al., 1997; Doolan et al., 1997; Threlkeld et al., 1997) have demonstrated that highly cross-reactive peptides are almost always recognized as epitopes. Given these

results, and in light of the limited immunogenicity data available, the use of B7-supertype binding affinity as a selection criterion was deemed appropriate.

Similarly, there is little immunogenicity data regarding A1- and A24-restricted peptides. One preferred CTL epitope, 1069.04, has been reported to be recognized in recall responses from acute HBV patients. As discussed in the preceding paragraph, a high percentage of the peptides with binding affinities <100 nM are found to be immunogenic. For this reason, all A1 and A24 peptides with binding affinities <100 nM were considered as preferred CTL epitopes. Using this selection criterion, 3 A1-restricted and 6 A24-restricted peptides are identified as candidate epitopes. Further analysis found that 3 core-derived peptides bound A24 with intermediate affinity. Since relatively few core epitopes were identified during the course of this study, the intermediate A24 binding core peptides were also included in the set of preferred epitopes to provide a greater degree of redundancy in antigen coverage.

The list of preferred HBV-derived HTL epitopes is summarized in Table XXXVII. The set of HTL epitopes includes 12 DR supermotif binding peptides and 4 DR3 binding peptides. The bulk of the HTL epitopes are derived from polymerase; 2 envelope and 2 core derived epitopes are also included in the set of preferred HTL epitopes. The total estimated population coverage represented by the panel of HTL epitopes is in excess of 91% in each of five major ethnic groups (Table XXXVIII)

Example 7: Recognition Of Generation Of Endogenous Processed Antigens After Priming

This example determines that CTL induced by native or analogued peptide epitopes identified and selected as described in Examples 1-5 recognize endogenously synthesized, i.e., native antigens.

Effector cells isolated from transgenic mice that are immunized with peptide epitopes as in Example 3 are re-stimulated *in vitro* using peptide-coated stimulator cells. Six days later, effector cells are assayed for cytotoxicity and the cell lines that contain peptide-specific cytotoxic activity are further re-stimulated. An additional six days later, these cell lines are tested for cytotoxic activity on ^{51}Cr labeled 3A4-721.221-A11/K^b target cells, in the absence or presence of peptide, and also tested on ^{51}Cr labeled target cells bearing the endogenously synthesized antigen, *e.g.*, cells that are stably transfected with HBV expression vectors.

The result will demonstrate that CTL lines obtained from animals primed with peptide epitope recognize endogenously synthesized HBV antigen.

Example 8: Activity Of CTL-HTL Conjugated Epitopes In Transgenic Mice

This example illustrates the induction of CTLs in transgenic mice by use of an HBV CTL/HTL peptide conjugate. An analagous study may be found in Oseroff *et al.* *Vaccine* 16:823-833 (1998). The peptide composition can comprise multiple CTL and/or HTL epitopes. Such a peptide composition can comprise a lipidated HTL epitope conjugated to a preferred CTL epitope containing, for example, an A11 motif or an analog of that epitope.

Lipopeptides are prepared by coupling the appropriate fatty acid to the amino terminus of the resin bound peptide. A typical procedure is as follows: A dichloromethane solution of a four-fold excess of a pre-formed symmetrical anhydride of the appropriate fatty acid is added to the resin and the mixture is allowed to react for two hours. The resin is washed with dichloromethane and dried. The resin is then treated with trifluoroacetic acid in the presence of appropriate scavengers [e.g. 5% (v/v) water] for 60 minutes at 20°C. After evaporation of excess trifluoroacetic acid, the crude peptide is washed with diethyl ether, dissolved in methanol and precipitated by the addition of water. The peptide is collected by filtration and dried.

Preparation of peptides for immunization: Peptide compositions are typically resuspended in DMSO at a concentration of 20 mg/ml. Before use, peptides are prepared at the required concentration by dilution in saline or the appropriate medium.

Immunization procedures: A11/K^b mice, which are transgenic for the human HLA A11 allele, are primed subcutaneously (base of the tail) with 0.1 ml of peptide conjugate formulated in saline, or DMSO/saline. Seven days after priming, splenocytes obtained from these animals are restimulated with syngeneic irradiated LPS-activated lymphoblasts coated with peptide.

Media:

a. RPMI-1640 supplemented with 10% fetal calf serum (FCS) 2 mM Glutamine, 50 µg/ml Gentamicin and $5x10^{-5}$ M 2-mercaptoethanol serves as culture medium

b. RPMI-1640 containing 25 mM HEPES buffer and supplemented with 2% (FCS) is used as cell washing medium.

Cell lines: The 3A4-721.221-A11/K^b cell line is used as target cells. This cell line is an EBV transformed cell line that was mutagenized and selected to be Class I negative which was transfected with an HLA-A11/K^b gene.

LPS-activated lymphoblasts: Splenocytes obtained from transgenic mice are resuspended at a concentration of 1-1.5x10^6/ml in culture medium supplemented with 25 μg/ml LPS and 7 μg/ml dextran sulfate in 75 cm^2 tissue culture flasks. After 72 hours at 37°C, the lymphoblasts are collected for use by centrifugation.

Peptide coating of lymphoblasts: Peptide coating of the LPS activated lymphoblasts is achieved by incubating 30x10^6 irradiated (3000 rads) lymphoblasts with 100 μg of peptide in 1 ml of R10 medium for 1 hr at 37°C. Cells are then washed once and resuspended in culture medium at the desired concentration.

In vitro CTL activation: One week after priming, spleen cells (30x10^6 cells/flask) are co-cultured at 37°C with syngeneic, irradiated (3000 rads), peptide coated lymphoblasts (10x10^6 cells/flask) in 10 ml of culture medium/T25 flask. After six days, the effector cells are harvested and assayed for cytotoxic activity.

Assay for cytotoxic activity: Target cells (1.0-1.5x10^6) are incubated at 37°C in the presence of 200 μl of sodium ^{51}Cr chromate. After 60 minutes, cells are washed three times and resuspended in R10 medium. Peptide is added where required at a concentration of 1 μg/ml. For the assay, 104 ^{51}Cr-labeled target cells are added to different concentrations of effector cells (final volume of 200 μl) in U-bottom 96-well plates. After a 6 hour incubation period at 37°C, a 0.1 ml aliquot of supernatant is removed from each well and radioactivity is determined in a Micromedic automatic gamma counter. The percent specific lysis is determined by the formula: percent specific release = 100 x (experimental release - spontaneous release)/(maximum release - spontaneous release). To facilitate comparison between separate CTL assays run under the same conditions, % ^{51}Cr release data is expressed as lytic units/10^6 cells. One lytic unit is arbitrarily defined as the number of effector cells required to achieve 30% lysis of 10,000 target cells in a 6 hour ^{51}Cr release assay. To obtain specific lytic units/10^6, the lytic units/10^6 obtained in the absence of peptide is subtracted from the lytic units/10^6 obtained in the presence of peptide. For example, if 30% ^{51}Cr release is obtained at the E:T of 50:1 (i.e., 5x10^5 effector cells for 10,000 targets) in the absence of peptide and 5:1 (i.e., 5x10^4 effector cells for 10,000 targets) in the presence of peptide, the specific lytic units would be: (1x10^6(5x10^4)-(1x10^6(5x10^5) = 18LU/10^6.

The results are analyzed to assess the magnitude of the CTL responses of animals injected with the immunogenic CTL/HTL conjugate vaccine preparation and are compared to the magnitude of the CTL response achieved using the CTL epitope as outlined in Example 3. Analyses similar to this may be performed to evaluate the immunogenicity of peptide conjugates containing multiple CTL epitopes and/or multiple HTL epitopes. In accordance with these procedures it is found that a CTL response is induced, and concomitantly that an HTL repsonse is induced upon administration of such compositions.

Example 9. Selection of CTL and HTL epitopes for inclusion in an HBV-specific vaccine.

This example illustrates the procedure for the selection of peptide epitopes for vaccine compositions of the invention.

The following principles are utilized when selecting an array of epitopes for inclusion in a polyepitopic composition, or for selecting epitopes to be included in a vaccine composition and/or to be encoded by a minigene. Each of the following principles are balanced in order to make the selection.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with HBV clearance. For HLA Class I this includes 3-4 epitopes that come from at least one antigen of HBV. In other words, it has been observed that in patients who spontaneously clear HBV, that they had generated an immune response to at least 3 epitopes on at least one HBV antigen. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one HBV antigen.

2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, or for Class II an IC_{50} of 1000 nM or less.

3.) Sufficient supermotif bearing peptides, or a sufficient array of allele-specific motif bearing peptides, are selected to give broad population coverage. For example, epitopes are selected to provide at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, is employed to assess population coverage.

4.) When selecting epitopes for HBV antigens it is often preferable to select native epitopes. Therefore, of particular relevance for infectious disease vaccines, are epitopes referred to as "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A peptide comprising "transcendent nested epitopes" is a peptide that has both HLA class I and HLA class II epitopes in it.

When providing nested epitopes, a sequence that has the greatest number of epitopes per provided sequence is provided. A limitation on this principle is to avoid providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a longer peptide sequence, such as a sequence comprising nested epitopes, the sequence is screened in order to insure that it does not have pathological or other deleterious biological properties.

5.) When creating a minigene, as disclosed in greater detail in the Example 9, an objective is to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same as those employed when selecting a peptide comprising nested epitopes. Thus, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is an actual binding epitope, as predicted, e.g., by motif analysis. Junctional epitopes are to be avoided because the recipient may generate an immune response to that epitope. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

Peptide epitopes for inclusion in vaccine compositions are, for example, selected from those listed in Table XXXVIIa and b. A vaccine composition comprised of selected peptides, when administered, is safe, efficacious, and elicits an immune response similar in magnitude of an immune response that clears an acute HBV infection.

Example 10: Construction of Minigene Multi-Epitope DNA Plasmids

This example provides an illustration of the construction of a minigene expression plasmid. Minigene plasmids may, of course, contain various configurations of CTL and/or HTL epitopes or epitope analogs as described herein. Expression plasmids have been constructed and evaluated as described, for example, in U.S.S.N. 60/085,751 filed 5/15/98 and U.S.S.N. 09/078,904 filed 5/13/98. An example of such a plasmid is shown

in Figure 2, which illustrates the orientation of HBV epitopes in minigene constructs. Such a plasmid may, for example, also include multiple CTL and HTL peptide epitopes.

A minigene expression plasmid may include multiple CTL and HTL peptide epitopes. In the present example, HLA-A2, -A3, -B7 supermotif-bearing peptide epitopes and HLA-A1 and -A24 motif-bearing peptide epitopes are used in conjunction with DR supermotif-bearing epitopes and/or DR3 epitopes (Figure 2). Preferred epitopes are identified, for example, in Tables XXVI-XXXIII, HLA class I supermotif or motif-bearing peptide epitopes derived from multiple HBV antigens, *e.g.*, the core, polymerase, envelope and X proteins, are selected such that multiple supermotifs/motifs are represented to ensure broad population coverage. Similarly, HLA class II epitopes are selected from multiple HBV antigens to provide broad population coverage, *i.e.* both HLA DR-1-4-7 supermotif-bearing epitopes and HLA DR-3 motif-bearing epitopes are selected for inclusion in the minigene construct. The selected CTL and HTL epitopes are then incorporated into a minigene for expression in an expression vector.

This example illustrates the methods to be used for construction of such a minigene-bearing expression plasmid. Other expression vectors that may be used for minigene compositions are available and known to those of skill in the art.

The minigene DNA plasmid contains a consensus Kozak sequence and a consensus murine kappa Ig-light chain signal sequence followed by a string of CTL and/or HTL epitopes selected in accordance with principles disclosed herein.

Overlapping oligonucleotides, for example eight oligonucleotides, averaging approximately 70 nucleotides in length with 15 nucleotide overlaps, are synthesized and HPLC-purified. The oligonucleotides encode the selected peptide epitopes as well as appropriate linker nucleotides. The final multiepitope minigene is assembled by extending the overlapping oligonucleotides in three sets of reactions using PCR. A Perkin/Elmer 9600 PCR machine is used and a total of 30 cycles are performed using the following conditions: 95°C for 15 sec, annealing temperature (5° below the lowest calculated Tm of each primer pair) for 30 sec, and 72°C for 1 min.

For the first PCR reaction, 5 μg of each of two oligonucleotides are annealed and extended: Oligonucleotides 1+2, 3+4, 5+6, and 7+8 are combined in 100 μl reactions containing *Pfu* polymerase buffer (1x= 10 mM KCL, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-chloride, pH 8.75, 2 mM $MgSO_4$, 0.1% Triton X-100, 100 μg/ml BSA), 0.25 mM each dNTP, and 2.5 U of *Pfu* polymerase. The full-length dimer products are gel-purified, and two reactions containing the product of 1+2 and 3+4, and the product of 5+6 and 7+8 are

mixed, annealed, and extended for 10 cycles. Half of the two reactions are then mixed, and 5 cycles of annealing and extension carried out before flanking primers are added to amplify the full length product for 25 additional cycles. The full-length product is gel-purified and cloned into pCR-blunt (Invitrogen) and individual clones are screened by sequencing.

Example 11. The plasmid construct and the degree to which it induces immunogenicity.

The degree to which the plasmid construct prepared using the methodology outlined in Example 9 is able to induce immunogenicity is evaluated through *in vivo* injections into transgenic mice and *in vitro* culture of CTL and HTL, which are subsequently analysed using cytotoxicity and proliferation assays, respectively, as detailed *e.g.,* in U.S.S.N. 09/311,784 filed 5/13/99 and Alexander *et al.*, *Immunity* 1:751-761, 1994. To assess the capacity of the pMin minigene construct to induce CTLs *in vivo*, HLA-A11/K^b transgenic mice, for example, are immunized intramuscularly with 100 μg of plasmid cDNA. As a means of comparing the level of CTLs induced by DNA immunization, a control group of animals is also immunized with an actual peptide composition that comprises multiple epitopes synthesized as a single polypeptide as they would be encoded by the minigene.

Splenocytes from immunized animals are stimulated twice with each of the respective compositions (peptide epitopes encoded in the minigene or the polyepitopic peptide), then assayed for peptide-specific cytotoxic activity in a ^{51}Cr release assay. The results indicate the magnitude of the CTL response directed against the A3-restricted epitope, thus indicating the *in vivo* immunogenicity of the minigene vaccine and polyepitopic vaccine. It is, therefore, found that the minigene elicits immune responses directed toward the HLA-A3 supermotif peptide epitopes as does the polyepitopic peptide vaccine. Such an analysis is also performed using other HLA-A2 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A2 and HLA-B7 motif or supermotif epitopes.

To assess the capacity of a class II epitope encoding minigene to induce HTLs *in vivo*, I-A^b restricted mice, for example, are immunized intramuscularly with 100 μg of plasmid DNA. As a means of comparing the level of HTLs induced by DNA immunization, a group of control animals is also immunized with an actual peptide composition emulsified in complete Freund's adjuvant.

CD4+ T cells, *i.e.* HTLs, are purified from splenocytes of immunized animals and stimulated with each of the respective compositions (peptides encoded in the minigene). The HTL response is measured using a ^{3}H-thymidine incorporation proliferation assay, (*see, e.g.*, Alexander et al. Immunity 1:751-761, 1994). the results indicate the magnitude of the HTL response , thus demonstrating the *in vivo* immunogenicity of the minigene.

Example 12: Peptide Composition for Prophylactic Uses

Vaccine compositions of the present invention are used to prevent HBV infection in persons who are at risk for such an infection. For example, a polyepitopic peptide epitope composition containing multiple CTL and HTL epitopes such as those selected in Examples 9 and/or 10, which are also selected to target greater than 80% of the population, is administered to individuals at risk for HBV infection. The composition is provided as a single lipidated polypeptide that encompasses multiple epitopes. The vaccine is administered in an aqueous carrier comprised of Freunds Incomplete Adjuvant. The dose of peptide for the initial immunization is from about 500 to about 50,000 μg for a 70 kg patient. The initial administration of vaccine is followed by booster dosages at 4 weeks followed by evaluation of the magnitude of the immune response in the patient by techniques that determine the presence of epitope-specific CTL populations in a PBMC sample. Additional booster doses are administered as required. The composition is found to be both safe and efficacious as a prophylaxis against HBV infection.

Alternatively, the polyepitopic peptide composition can be administered as a nucleic acid in accordance with methodologies known in the art and disclosed herein.

Example 13: Polyepitopic Vaccine Compositions Derived from Native HBV Sequences

A native HBV polyprotein sequence is screened, preferably using computer algorithms defined for each class I and/or class II supermotif or motif, to identify "relatively short" regions of the polyprotein that comprise multiple epitopes. This relatively short sequence that contains multiple distinct, even overlapping, epitopes is selected and used to generate a minigene construct. The construct is engineered to express the peptide, which corresponds to the native protein sequence. The "relatively short" peptide is less than 250 amino acids in length, preferably less than 100 amino acids in length, and more preferably less than 75 or 50 amino acids in length. The protein sequence of the vaccine composition is selected because it has maximal number of epitopes contained within the sequence. As noted herein, epitope motifs may be

overlapping (*i.e.,* frame shifted relative to one another) with frame shifted overlapping epitopes, *e.g.* two 9-mer epitopes can be present in a 10 amino acid peptide. Such a vaccine composition is administered for therapeutic or prophylactic purposes.

The vaccine composition will preferably include, for example, three CTL epitopes and at least one HTL epitope from the source antigen. This polyepitopic native sequence is administered either as a peptide or as a nucleic acid sequence which encodes the peptide. Alternatively, an analog can be made of this native sequence, whereby one or more of the epitopes comprise substitutions that alter the cross-reactivity and/or binding affinity properties of the polyepitopic peptide.

The embodiment of this example provides for the possibility that an as yet undiscovered aspect of immune system processing will apply to the native nested sequence and thereby facilitate the production of therapeutic or prophylactic immune response-inducing vaccine compositions. Additionally such an embodiment provides for the possibility of motif-bearing epitopes for an HLA makeup that is presently unknown. Furthermore, this embodiment (absent analogs) directs the immune response to peptide sequences that are present in native HBV antigens. Lastly, the embodiment provides an economy of scale when producing nucleic acid vaccine compositions.

Related to this embodiment, computer programs can be derived which identify, in a target sequence, the greatest number of epitopes per sequence length.

Example 14. Polyepitopic Vaccine Compositions Directed To Multiple Diseases

The HBV peptide epitopes of the present invention are used in conjunction with peptide epitopes from target antigens related to one or more other diseases, to create a vaccine composition that is useful for the prevention or treatment of HBV as well as another disease. Examples of other diseases include, but are not limited to, HIV, HCV, and HPV.

For example, a polyepitopic peptide composition comprising multiple CTL and HTL epitopes that target greater than 98% of the population may be created for administration to individuals at risk for both HBV and HIV infection. The composition can be provided as a single polypeptide that incorporates the multiple epitopes from the various disease-associated sources, or can be administered as a composition comprising one or more discrete epitopes.

Example 15. Use of peptides to evaluate an immune response

Peptides of the invention may be used to analyze an immune response for the presence of specific CTL populations corresponding to HBV. Such an analysis may be performed as described by Ogg *et al., Science* 279:2103-2106, 1998. In the following example, peptides in accordance with the invention are used as a reagent for diagnostic or prognostic purposes, not as an immunogen.

In this example highly sensitive human leukocyte antigen tetrameric complexes ("tetramers") may be used for a cross-sectional analysis of, for example, HBV Env-specific CTL frequencies from untreated HLA A*0201-positive indiviuals at different stages of infection using an HBV Env peptide containing an A2.1 extended motif. Tetrameric complexes are synethesized as described (Musey *et al., N. Engl. J. Med.* 337:1267, 1997). Briefly, purified HLA heavy chain (A2.1 in this example) and β2-microglobulin are synthesized by means of a prokaryotic expression system. The heavy chain is modified by deletion of the transmembrane-cytosolic tail and COOH-terminal addition of a sequence containing a BirA enzymatic biotinylation site. The heavy chain, β2-microglobulin, and peptide are refolded by dilution. The 45-kD refolded product is isolated by fast protein liquid chromatography and then biotinylated by BirA in the presence of biotin (Sigma, St. Louis, Missouri), adenosine 5'triphosphate and magnesium. Streptavidin-phycoerythrin conjugate is added in a 1:4 molar ratio, and the tetrameric product is concentrated to 1 mg/ml. The resulting product is referred to as tetramer-phycoerythrin.

For the analysis of patient blood samples, approximately one million PBMCs are centrifuged at 300g for 5 minutes and resuspended in 50 ul of cold phosphate-buffered saline. Tri-color analysis is performed with the tetramer-phycoerythrin, along with anti-CD8-Tricolor, and anti-CD38. The PBMCs are incubated with tetramer and antibodies on ice for 30 to 60 min and then washed twice before formaldehyde fixaation. Gates are applied to contain >99.98% of control samples. Controls for the tetramers include both A*0201-negative individuals and A*0201-positive uninfected donors. The percentage of cells stained with the tetramer is then determined by flow cytometry. The results indicate the number of cells in the PBMC sample that contain epitope-restricted CTLs, thereby readily indicating the stage of infection with HBV or the status of exposure to HBV or to a vaccine that elicits a protective response.

Example 16: Use of Peptide Epitopes to Evaluate Recall Responses

The peptide epitopes of the invention are used as reagents to evaluate T cell responses such as acute or recall responses, in patients. Such an analysis may be performed on patients who have recovered from infection or who are chronically infected with HBV or who have been vaccinated with an HBV vaccine.

For example, the class I restricted CTL response of persons at risk for HBV infection who have been vaccinated may be analyzed. The vaccine may be any HBV vaccine. PBMC are collected from vaccinated individuals and HLA typed. Appropriate peptide reagents that, are highly conserved and, optimally, bear supermotifs to provide cross-reactivity with multiple HLA supertype family members are then used for analysis of samples derived from individuals who bear that HLA type.

PBMC from vaccinated individuals are separated on Ficoll-Histopaque density gradients (Sigma Chemical Co., St. Louis, MO), washed three times in HBSS (GIBCO Laboratories), resuspended in RPMI-1640 (GIBCO Laboratories) supplemented with L-glutamine (2mM), penicillin (50U/ml), streptomycin (50 μg/ml), and Hepes (10mM) containing 10% heat-inactivated human AB serum (complete RPMI) and plated using microculture formats. Synthetic peptide is added at 10 μg/ml to each well and recombinant HBc Ag is added at 1 μg/ml to each well as a source of T cell help during the first week of stimulation.

In the microculture format, 4 x 10^5 PBMC are stimulated with peptide in 8 replicate cultures in 96-well round bottom plate in 100 μl/well of complete RPMI. On days 3 and 10, 100 ml of complete RPMI and 20 U/ml final concentration of rIL-2 are added to each well. On day 7 the cultures are transferred into a 96-well flat-bottom plate and restimulated with peptide, rIL-2 and 10^5 irradiated (3,000 rad) autologous feeder cells. The cultures are tested for cytotoxic activity on day 14. A positive CTL response requires two or more of the eight replicate cultures to display greater than 10% specific ^{51}Cr release, based on comparison with uninfected control subjects as previously described (Rehermann, *et al., Nature Med.* 2:1104,1108, 1996; Rehermann *et al., J. Clin. Invest.* 97:1655-1665, 1996; and Rehermann *et al. J. Clin. Invest.* 98:1432-1440, 1996).

Target cell lines are autologous and allogeneic EBV-transformed B-LCL that are either purchased from the American Society for Histocompatibility and Immunogenetics (ASHI, Boston, MA) or established from the pool of patients as described (Guilhot, *et al. J. Virol.* 66:2670-2678, 1992).

Cytotoxicity assays are performed in the following manner. Target cells consist of either allogeneic HLA-matched or autologous EBV-transformed B lymphoblastoid cell line that are incubated overnight with synthetic peptide at 10 μM and labeled with 100 μCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 1 hour after which they are washed four times with HBSS. Cytolytic activity is determined in a standard 4-h, split well ^{51}Cr release assay using U-bottomed 96 well plates containing 3,000 targets/well. Stimulated PBMC are tested at E/T ratios of 20-50:1 on day 14. Percent cytotoxicity is determined from the formula: 100 x [(experimental release-spontaneous release)/maximum release-spontaneous release)]. Maximum release is determined by lysis of targets by detergent (2% Triton X-100; Sigma Chemical Co., St. Louis, MO). Spontaneous release is <25% of maximum release for all experiments.

The results of such an analysis will indicate to what extent HLA-restricted CTL populations have been stimulated with the vaccine. Of course, this protocol can also be used to monitor prior HBV exposure.

The class II restricted HTL responses may also be analyzed. Purified PBMC are cultured in a 96-well flat bottom plate at a density of $1.5x10^5$ cells/well and are stimulated with 10 μg/ml synthetic peptide, whole antigen, or PHA. Cells are routinely plated in replicates of 4-6 wells for each condition. After seven days of culture, the medium is removed and replaced with fresh medium containing 10U/ml IL-2. Two days later, 1 μCi ^3H-thymidine is added to each well and incubation is continued for an additional 18 hours. Cellular DNA is then harvested on glass fiber mats and analyzed for ^3H-thymidine incorporation. Antigen-specific T cell proliferation is calculated as the ratio of ^3H-thymidine incorporation in the presence of antigen divided by the ^3H-thymidine incorporation in the absence of antigen.

The results of such an analysis will indicate to what extent HLA-restricted HTL populations have been stimulated with a vaccine or prior exposure to HBV.

Example 17: Induction Of Specific CTL Response In Humans

A human clinical trial for an immunogenic composition comprising HBV CTL and HTL epitopes of the invention is set up as an IND Phase I, dose escalation study (5, 50 and 500 μg) and carried out as a randomized, double-blind, placebo-controlled trial. Such a trial is designed, for example, as follows:

A total of about 27 subjects are enrolled and divided into 3 groups:

Group I: 3 subjects are injected with placebo and 6 subjects are injected with 5 μg of peptide composition;

Group II: 3 subjects are injected with placebo and 6 subjects are injected with 50 μg peptide composition;

Group III: 3 subjects are injected with placebo and 6 subjects are injected with 500 μg of peptide composition.

After 4 weeks following the first injection, all subjects receive a booster inoculation at the same dosage.

The endpoints measured in this study relate to the safety and tolerability of the peptide composition as well as its immunogenicity. Cellular immune responses to the peptide composition are an index of the intrinsic activity of this the peptide composition, and can therefore be viewed as a measure of biological efficacy. The following summarize the clinical and laboratory data that relate to safety and efficacy endpoints.

Safety: The incidence of adverse events is monitored in the placebo and drug treatment group and assessed in terms of degree and reversibility.

Evaluation of Vaccine Efficacy: For evaluation of vaccine efficacy, subjects are bled before and after injection. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

Thus, the vaccine is found to be both safe and efficacious.

Example 18: Phase II Trials In Patients Infected With HBV

Phase II trials are performed to study the effect of administering the CTL-HTL peptide compositions to patients (male and female) having chronic HBV infection. A main objective of the trials is to determine an effective dose and regimen for inducing CTLs in chronically infected HBV patients, to establish the safety of inducing a CTL response in these patients, and to see to what extent activation of CTLs improves the clinical picture of chronically infected CTL patients, as manifested by a transient flare in alanine aminotransferase (ALT), normalization of ALT, and reduction in HBV DNA. Such a study is designed, for example, as follows:

The studies are performed in multiple centers in the U.S. and Canada. The trial design is an open-label, uncontrolled, dose escalation protocol wherein the peptide composition is administered as a single dose followed six weeks later by a single booster

shot of the same dose. The dosages are 50, 500 and 5,000 micrograms per injection. Drug-associated adverse effects are recorded.

There are three patient groupings. The first group is injected with 50 micrograms of the peptide composition and the second and third groups with 500 and 5,000 micrograms of peptide composition, respectively. The patients within each group range in age from 21-65 and include both males and females. The patients represent diverse ethnic backgrounds. All of them are infected with HBV for over five years and are HIV, HCV and HDV negative, but have positive levels of HBe antigen and HBs antigen.

The magnitude and incidence of ALT flares and the levels of HBV DNA in the blood are monitored to assess the effects of administering the peptide compositions. The levels of HBV DNA in the blood are an indirect indication of the progress of treatment. The vaccine composition is found to be both safe and efficacious in the treatment of chronic HBV infection.

The examples herein are provided to illustrate the invention but not to limit its scope. For example, the human terminology for the Major Histocompatibility Complex, namely HLA, is used throughout this document. It is to be appreciated that these principles can be extended to other species as well. Moreover, peptide epitopes have been disclosed in the related application U.S.S.N. 08/820,360, which was previously incorporated by reference. Thus, other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent application cited herein are hereby incorporated by reference for all purposes.

TABLE I

SUPERMOTIFS	POSITION 2 (Primary Anchor)	POSITION 3 (Primary Anchor)	POSITION C Terminus (Primary Anchor)
A1	**TI***LVMS*		**FWY**
A2	**LIVM***ATQ*		**IV***MATL*
A3	**VSMA***TLI*		**RK**
A24	**YF***WIVLMT*		**FI***YWLM*
B7	**P**		**VILF***MWYA*
B27	**RHK**		**FYL***WMIVA*
B44	**E***D*		**FWYLIMVA**
B58	**ATS**		**FWY***LIVMA*
B62	**QL***IVMP*		**FWY***MIVLA*
MOTIFS			
A1	**TSM**		**Y**
A1		**DE***AS*	**Y**
A2.1	**LM***VQIAT*		**V***LIMAT*
A3	**LMVISATF***CGD*		**KYR***HFA*
A11	**VTMLISAGN***CDF*		**K***RYH*
A24	**YFW***M*		**FLIW**
A*3101	**MVT***ALIS*		**R***K*
A*3301	**MVALF***IST*		**RK**
A*6801	**AVT***MSLI*		**RK**
B*0702	**P**		**LMF***WYAIV*
B*3501	**P**		**LMFWY***IVA*
B51	**P**		**LIVF***WYAM*
B*5301	**P**		**IMFWY***ALV*
B*5401	**P**		**ATIV***LMFWY*

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE Ia

SUPERMOTIFS	POSITION	POSITION	POSITION
	2 (Primary Anchor)	3 (Primary Anchor)	C Terminus (Primary Anchor)
A1	**TL***VMS*		**FWY**
A2	*VQAT*		**V***LIMAT*
A3	**VSMA***TLI*		**RK**
A24	**YF***WIVLMT*		**FI***YWLM*
B7	**P**		**VILF***MWYA*
B27	**RHK**		**FYL***WMIVA*
B58	**ATS**		**FWY***LIVMA*
B62	**QL***IVMP*		**FWY***MIVLA*
MOTIFS			
A1	**TSM**		**Y**
A1		**DE***AS*	**Y**
A2.1	*VQAT**		**V***LIMAT*
A3.2	**LMVISATF***CGD*		**KYR***HF*
A11	**VTMLISAGN***CDF*		**K***RH*
A24	**YFW**		**FLIW**

*If 2 is V, or Q, the C-term is not L

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE II

		POSITION								
		1	2	3	4	5	6	7	8	C-terminus
SUPERMOTIFS										
A1			1° Anchor TI*LVMS*							1° Anchor FWY
A2			1° Anchor LIVM*ATQ*							1° Anchor LIVMAT
A3	preferred		1° Anchor VSMA*TLI*	YFW (4/5)			YFW (3/5)	YFW (4/5)	P (4/5)	1°Anchor RK
	deleterious	DE (3/5); P (5/5)		DE (4/5)						
A24			1° Anchor YF*WIVLM* *T*							1° Anchor FI*YWLM*
B7	preferred	FWY (5/5) LIVM (3/5)	1°Anchor P	FWY (4/5)					FWY (3/5)	1°Anchor VILF*MWYA*
	deleterious	DE (3/5); P(5/5); G(4/5); A(3/5); QN (3/5)				DE (3/5)	G (4/5)	QN (4/5)	DE (4/5)	
B27			1° Anchor RHK							1° Anchor FYL*WMIVA*
B44			1° Anchor E*D*							1° Anchor FWYLIMVA
B58			1° Anchor ATS							1° Anchor FWY*LIVMA*
B62			1° Anchor QL*IVMP*							1° Anchor FWY*MIVLA*

		POSITION								
		1	2	3	4	5	6	7	8	C-terminus
MOTIFS										
A1 9-mer	preferred	GFYW	1°Anchor STM	DEA	YFW		P	DEQN	YFW	1°Anchor Y
	deleterious	DE		RHKLIVM P	A	G	A			
A1 9-mer	preferred	GRHK	ASTCLIV M	1°Anchor DE*AS*	GSTC		ASTC	LIVM	DE	1°Anchor Y
	deleterious	A	RHKDEPY FW		DE	PQN	RHK	PG	GP	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A1 10-mer	peferred	YFW	1°Anchor STM	DEAQN	A	YFWQN		PASTC	GDE	P	1°Anchor Y
	deleterious	GP		RHKGLIVM	DE	RHK	QNA	RHKYFW	RHK	A	
A1 10-mer	preferred	YFW	STCLIVM	1°Anchor DE*AS*	A	YFW		PG	G	YFW	1°Anchor Y
	deleterious	RHK	RHKDEPYFW			P	G		PRHK	QN	
A2.1 9-mer	preferred	YFW	1°Anchor LM*IVQAT*	YFW	STC	YFW		A	P	1°Anchor V*LIMAT*	
	deleterious	DEP		DERKH			RKH	DERKH			
A2.1 10-mer	preferred	AYFW	1°Anchor LM*IVQAT*	LVIM	G		G		FYWLVIM		1°Anchor V*LIMAT*
	deleterious	DEP		DE	RKHA	P		RKH	DERKH	RKH	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A3	preferred	RHK	1°Anchor LMVISAT F*CGD*	YFW	PRHKYFW	A	YFW		P	1°Anchor KYR*HFA*	
	deleterious	DEP		DE							
A11	preferred	A	1°Anchor VTLMISA GN*CDF*	YFW	YFW	A	YFW	YFW	P	1°Anchor *KRYH*	
	deleterious	DEP						A	G		
A24 9-mer	preferred	YFWRHK	1°Anchor YFW*M*		STC			YFW	YFW	1°Anchor FLIW	
	deleterious	DEG		DE	G	QNP	DERHK	G	AQN		
A24 10-mer	preferred		1°Anchor YFW*M*		P	YFWP		P			1°Anchor FLIW
	deleterious			GDE	QN	RHK	DE	A	QN	DEA	
A3101	preferred	RHK	1°Anchor MVT*ALIS*	YFW	P		YFW	YFW	AP	1°Anchor R*K*	
	deleterious	DEP		DE		ADE	DE	DE	DE		

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A3301	preferred		1°Anchor MVALF*IS T*	YFW				AYFW		1°Anchor RK	
	deleterious	GP		DE							
A6801	preferred	YFWSTC	1°Anchor AVT*MSLI*			YFWLIV M		YFW	P	1°Anchor RK	
	deleterious	GP		DEG		RHK			A		
B0702	preferred	RHKFWY	1°Anchor P	RHK		RHK	RHK	RHK	PA	1°Anchor LMF*WYAIV*	
	deleterious	DEQNP		DEP	DE	DE	GDE	QN	DE		
B3501	preferred	FWYLIVM	1°Anchor P	FWY				FWY		1°Anchor LMFWY*IVA*	
	deleterious	AGP				G	G				
B51	preferred	LIVMFWY	1°Anchor P	FWY	STC	FWY		G	FWY	1°Anchor LIVF*WYAM*	
	deleterious	AGPDERHKSTC				DE	G	DEQN	GDE		

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
B5301	preferred	LIVMFWY	1°Anchor P	FWY	STC	FWY		LIVMFWY	FWY	1°Anchor IMFWY*ALV*	
	deleterious	AGPQN					G	RHKQN	DE		
B5401	preferred	FWY	1°Anchor P	FWYLIVM		LIVM		ALIVM	FWYAP	1°Anchor ATIV*LMFW Y*	
	deleterious	GPQNDE		GDESTC		RHKDE	DE	QNDGE	DE		

Italicized residues indicate less preferred or "tolerated" residues.
The information in Table II is specific for 9-mers unless otherwise specified.

TABLE III

MOTIFS		POSITION 1° anchor 1	2	3	4	5	1° anchor 6	7	8	9
DR4	preferred	FMY*LIVW*	M	T		I	VST*CPALIM*	MH		MH
	deleterious				W			R		WDE
DR1	preferred	MF*LIVWY*			PAMQ		VMAT*SPLIC*	M		AVM
	deleterious		C	CH	FD	CWD		GDE	D	
DR7	preferred	MF*LIVWY*	M	W	A		IVMSA*CTPL*	M		IV
	deleterious		C		G			GRD	N	G
DR Supermotif		MF*LIVWY*					VMSTA*CPLI*			

DR3 MOTIFS	1° anchor 1	2	3	1° anchor 4	5	1° anchor 6
motif a preferred	LIVMFY			D		
motif b preferred	LIVMFAY			DNQEST		KRH

Italicized residues indicate less preferred or "tolerated" residues.

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Table IV. HLA Class I Standard Peptide Binding Affinity.

ALLELE	STANDARD PEPTIDE	SEQUENCE	STANDARD BINDING AFFINITY (nM)
A*0101	944.02	YLEPAIAKY	25
A*0201	941.01	FLPSDYFPSV	5.0
A*0202	941.01	FLPSDYFPSV	4.3
A*0203	941.01	FLPSDYFPSV	10
A*0206	941.01	FLPSDYFPSV	3.7
A*0207	941.01	FLPSDYFPSV	23
A*6802	1141.02	FTQAGYPAL	40
A*0301	941.12	KVFPYALINK	11
A*1101	940.06	AVDLYHFLK	6.0
A*3101	941.12	KVFPYALINK	18
A*3301	1083.02	STLPETYVVRR	29
A*6801	941.12	KVFPYALINK	8.0
A*2401	979.02	AYIDNYNKF	12
B*0702	1075.23	APRTLVYLL	5.5
B*3501	1021.05	FPFKYAAAF	7.2
B51	1021.05	FPFKYAAAF	5.5
B*5301	1021.05	FPFKYAAAF	9.3
B*5401	1021.05	FPFKYAAAF	10

Table V. HLA Class II Standard Peptide Binding Affinity.

Allele	Nomenclature	Standard Peptide	Sequence	Binding Affinity (nM)
DRB1*0101	DR1	515.01	PKYVKQNTLKLAT	5.0
DRB1*0301	DR3	829.02	YKTIAFDEEARR	300
DRB1*0401	DR4w4	515.01	PKYVKQNTLKLAT	45
DRB1*0404	DR4w14	717.01	YARFQSQTTLKQKT	50
DRB1*0405	DR4w15	717.01	YARFQSQTTLKQKT	38
DRB1*0701	DR7	553.01	QYIKANSKFIGITE	25
DRB1*0802	DR8w2	553.01	QYIKANSKFIGITE	49
DRB1*0803	DR8w3	553.01	QYIKANSKFIGITE	1600
DRB1*0901	DR9	553.01	QYIKANSKFIGITE	75
DRB1*1101	DR5w11	553.01	QYIKANSKFIGITE	20
DRB1*1201	DR5w12	1200.05	EALIHQLKINPYVLS	298
DRB1*1302	DR6w19	650.22	QYIKANAKFIGITE	3.5
DRB1*1501	DR2w2β1	507.02	GRTQDENPVVHFFKNIV TPRTPPP	9.1
DRB3*0101	DR52a	511	NGQIGNDPNRDIL	470
DRB4*0101	DRw53	717.01	YARFQSQTTLKQKT	58
DRB5*0101	DR2w2β2	553.01	QYIKANSKFIGITE	20

The "Nomenclature" column lists the allelic designations used in Tables XIX and XX.

Table VI

HLA-supertype	Allele-specific HLA-supertype members	
	Verified[a]	Predicted[b]
A1	A*0101, A*2501, A*2601, A*2602, A*3201	A*0102, A*2604, A*3601, A*4301, A*8001
A2	A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, A*6901	A*0208, A*0210, A*0211, A*0212, A*0213
A3	A*0301, A*1101, A*3101, A*3301, A*6801	A*0302, A*1102, A*2603, A*3302, A*3303, A*3401, A*3402, A*6601, A*6602, A*7401
A24	A*2301, A*2402, A*3001	A*2403, A*2404, A*3002, A*3003
B7	B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, B*7801	B*1511, B*4201, B*5901
B27	B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, B*7301	B*2701, B*2707, B*2708, B*3802, B*3903, B*3904, B*3905, B*4801, B*4802, B*1510, B*1518, B*1503
B44	B*1801, B*1802, B*3701, B*4402, B*4403, B*4404, B*4001, B*4002, B*4006	B*4101, B*4501, B*4701, B*4901, B*5001
B58	B*5701, B*5702, B*5801, B*5802, B*1516, B*1517	
B62	B*1501, B*1502, B*1513, B*5201	B*1301, B*1302, B*1504, B*1505, B*1506, B*1507, B*1515, B*1520, B*1521, B*1512, B*1514, B*1519

a. Verified alleles includes alleles whose specificity has been determined by pool sequencing analysis, peptide binding assays, or by analysis of the sequences of CTL epitopes.

b. Predicted alleles are alleles whose specificity is predicted on the basis of B and F pocket structure to overlap with the supertype specificity.

TABLE VII

HBV A01 SUPER MOTIF(With binding information)

Conservancy	Freq.	Protein	Position	Sequence	String	A*0101
95	19	POL	521	AICSVVRRAF	XIXXXXXXF	
95	19	NUC	54	ALRQAILCW	XLXXXXXW	
80	16	ENV	108	AMQWNSTTF	XMXXXXXF	
100	20	POL	166	ASFCGSPY	XSXXXXY	
100	20	POL	166	ASFCGSPYSW	XSXXXXXXW	
90	18	NUC	19	ASKLCLGW	XSXXXXW	
85	17	NUC	19	ASKLCLGWLW	XSXXXXXXW	
80	16	POL	822	ASPLHVAW	XSXXXXW	
100	20	ENV	312	CIPIPSSW	XIXXXXW	
100	20	ENV	312	CIPIPSSWAF	XIXXXXXXF	
95	19	ENV	253	CLFLLVLLDY	XLXXXXXXXY	
95	19	ENV	239	CLRRFIIF	XLXXXXF	
75	15	ENV	239	CLRRFIIFLF	XLXXXXXXF	
95	19	POL	523	CSVVRRAF	XSXXXXF	
100	20	ENV	310	CTCIPIPSSW	XTXXXXXXW	
90	18	NUC	31	DIDPYKEF	XIXXXXF	11.1000
85	17	NUC	29	DLLDTASALY	XLXXXXXXY	
95	19	ENV	196	DSWWTSLNF	XSXXXXXF	
95	19	NUC	43	ELLSFLPSDF	XLXXXXXXF	
95	19	NUC	43	ELLSFLPSDFF	XLXXXXXXXF	
95	19	POL	374	ESRLVVDF	XSXXXXXF	
95	19	POL	374	ESRLVVDFSQF	XSXXXXXXXXF	
80	16	ENV	248	FILLLCLIF	XIXXXXXF	
80	16	ENV	246	FLFILLLCLIF	XLXXXXXXXXF	
95	19	ENV	256	FLLVLLDY	XLXXXXY	
95	19	POL	658	FSPTYKAF	XSXXXXXF	
90	18	X	63	FSSAGPCALRF	XSXXXXXXXXF	
100	20	ENV	333	FSWLSLLVPF	XSXXXXXXF	
95	19	POL	656	FTFSPTYKAF	XTXXXXXXF	
95	19	ENV	346	FVGLSPTVW	XVXXXXXXW	
95	19	POL	627	GLLGFAAPF	XLXXXXXF	
95	19	POL	509	GLSPFLLAQF	XLXXXXXXF	
85	17	NUC	29	GMDIDPYKEF	XMXXXXXXF	0.0017
95	19	NUC	123	GVWIRTPPAY	XVXXXXXXY	
75	15	POL	569	HLNPNKTKRW	XLXXXXXXW	
80	16	POL	491	HLYSHPIILGF	XLXXXXXXXF	
85	17	POL	715	HTAELLAACF	XTXXXXXXF	
95	19	NUC	52	HTALRQAILCW	XTXXXXXXXW	
100	20	POL	149	HTLWKAGILY	XTXXXXXXY	0.0300
100	20	ENV	249	ILLLCLIF	XLXXXXF	
80	16	POL	760	ILRGTSFVY	XLXXXXXY	0.0017
90	18	ENV	188	ILTIPQSLDSW	XLXXXXXXXW	
90	18	POL	625	IVGLLGFAAPF	XVXXXXXXXF	
80	16	POL	503	KIPMGVGLSPF	XIXXXXXXXF	
85	17	NUC	21	KLCLGWLW	XLXXXXW	
75	15	POL	108	KLIMPARF	XLXXXXF	
75	15	POL	108	KLIMPARFY	XLXXXXXY	0.0017
80	16	POL	610	KLPVNRPIDW	XLXXXXXXW	
85	17	POL	574	KTKRWGYSLNF	XTXXXXXXXF	
95	19	POL	55	KVGNFTGLY	XVXXXXXXY	0.0680
95	19	ENV	254	LIFLLVLLDY	XIXXXXXXXY	0.0084
100	20	POL	109	LIMPARFY	XIXXXXXY	
85	17	NUC	30	LLDTASALY	XLXXXXXXY	25.0000
80	16	POL	752	LLGCAANW	XLXXXXXW	
95	19	POL	628	LLGFAAPF	XLXXXXXF	
100	20	ENV	378	LLPIFFCLW	XLXXXXXXW	
100	20	ENV	378	LLPIFFCLWVY	XLXXXXXXXXY	
95	19	NUC	44	LLSFLPSDF	XLXXXXXF	
95	19	NUC	44	LLSFLPSDFF	XLXXXXXXXF	
90	18	POL	407	LLSSNLSW	XLXXXXXW	
95	19	ENV	175	LLVLQAGF	XLXXXXXF	
95	19	ENV	175	LLVLQAGFF	XLXXXXXXF	
100	20	ENV	338	LLVPFVQW	XLXXXXXW	
100	20	ENV	338	LLVPFVQWF	XLXXXXXXF	
85	17	NUC	100	LLWFHISCLTF	XLXXXXXXXXF	
95	19	NUC	45	LSFLPSDF	XSXXXXXF	
95	19	NUC	45	LSFLPSDFF	XSXXXXXXF	
95	19	POL	415	LSLDVSAAF	XSXXXXXXF	
95	19	POL	415	LSLDVSAAFY	XSXXXXXXXY	4.2000
100	20	ENV	336	LSLLVPFVQW	XSXXXXXXXW	
100	20	ENV	336	LSLLVPFVQWF	XSXXXXXXXXF	
95	19	X	53	LSLRGLPVCAF	XSXXXXXXXXF	

HBV A01 SUPER MOTIF(With binding information)

Conservancy	Freq.	Protein	Position	Sequence	String	A*0101
95	19	POL	510	LSPFLLAQF	XSXXXXXXF	
75	15	ENV	349	LSPTVWLSVIW	XSXXXXXXXXW	
85	17	POL	742	LSRKYTSF	XSXXXXXF	
85	17	POL	742	LSRKYTSFPW	XSXXXXXXXW	
75	15	ENV	16	LSVPNPLGF	XSXXXXXXF	
75	15	NUC	137	LTFGRETVLEY	XTXXXXXXXXY	
90	18	ENV	189	LTIPQSLDSW	XTXXXXXXXW	
90	18	ENV	189	LTIPQSLDSWW	XTXXXXXXXXW	
90	18	POL	404	LTNLLSSNLSW	XTXXXXXXXXW	
95	19	ENV	178	LVLQAGFF	XVXXXXXF	
100	20	ENV	339	LVPFVQWF	XVXXXXXF	
100	20	POL	377	LVVDFSQF	XVXXXXXF	0.0810
85	17	ENV	360	MMWYWGPSLY	XMXXXXXXXY	0.8500
75	15	X	103	MSTTDLEAY	XSXXXXXXY	
75	15	X	103	MSTTDLEAYF	XSXXXXXXXF	
95	19	POL	42	NLGNLNVSIPW	XLXXXXXXXXW	
90	18	POL	406	NLLSSNLSW	XLXXXXXXW	
95	19	POL	45	NLNVSIPW	XLXXXXXW	
75	15	ENV	15	NLSVPNPLGF	XLXXXXXXXF	0.0005
90	18	POL	738	NSVVLSRKY	XSXXXXXXY	0.0078
100	20	ENV	380	PIFFCLWVY	XIXXXXXXY	
100	20	ENV	314	PIPSSWAF	XIXXXXXF	
100	20	POL	124	PLDKGIKPY	XLXXXXXXY	0.0190
100	20	POL	124	PLDKGIKPYY	XLXXXXXXXY	0.1600
100	20	ENV	377	PLLPIFFCLW	XLXXXXXXXW	
95	19	ENV	174	PLLVLQAGF	XLXXXXXXF	
95	19	ENV	174	PLLVLQAGFF	XLXXXXXXXF	
80	16	POL	505	PMGVGLSPF	XMXXXXXXF	0.7700
85	17	POL	797	PTTGRTSLY	XTXXXXXXY	
75	15	ENV	351	PTVWLSVIW	XTXXXXXXW	
85	17	POL	612	PVNRPIDW	XVXXXXXW	
95	19	POL	685	QVFADATPTG	XVXXXXXXXXW	
90	18	POL	624	RIVGLLGF	XIXXXXXF	
75	15	POL	106	RLKLIMPARF	XLXXXXXXXF	
75	15	POL	106	RLKLIMPARFY	XLXXXXXXXXY	
95	19	POL	376	RLVVDFSQF	XLXXXXXXF	
90	18	POL	353	RTPARVTGGVF	XTXXXXXXXXF	
100	20	POL	49	SIPWTHKVGNF	XIXXXXXXXXF	
95	19	ENV	194	SLDSWWTSLNF	XLXXXXXXXXF	
95	19	POL	416	SLDVSAAF	XLXXXXXF	17.2000
95	19	POL	416	SLDVSAAFY	XLXXXXXXY	
100	20	ENV	337	SLLVPFVQW	XLXXXXXXW	
100	20	ENV	337	SLLVPFVQWF	XLXXXXXXXF	
95	19	X	54	SLRGLPVCAF	XLXXXXXXXF	
90	18	X	64	SSAGPCALRF	XSXXXXXXXF	
75	15	X	104	STTDLEAY	XTXXXXXY	
75	15	X	104	STTDLEAYF	XTXXXXXXF	
75	15	ENV	17	SVPNPLGF	XVXXXXXF	
90	18	POL	739	SVVLSRKY	XVXXXXXY	
85	17	POL	739	SVVLSRKYTSF	XVXXXXXXXXF	
90	18	ENV	190	TIPQSLDSW	XIXXXXXXW	
90	18	ENV	190	TIPQSLDSWW	XIXXXXXXXW	
100	20	POL	150	TLWKAGILY	XLXXXXXXY	0.0017
75	15	X	105	TTDLEAYF	XTXXXXXF	
85	17	POL	798	TTGRTSLY	XTXXXXXY	
80	16	NUC	16	TVQASKLCLGW	XVXXXXXXXXW	
75	15	ENV	352	TVWLSVIW	XVXXXXXW	
85	17	POL	741	VLSRKYTSF	XLXXXXXXF	
85	17	POL	741	VLSRKYTSFPW	XLXXXXXXXXW	
85	17	POL	740	VVLSRKYTSF	XVXXXXXXXF	
80	16	POL	759	WILRGTSF	XIXXXXXF	0.0023
80	16	POL	759	WILRGTSFVY	XIXXXXXXXY	
95	19	NUC	125	WIRTPPAY	XIXXXXXY	
80	16	POL	751	WLLGCAANW	XLXXXXXXW	
95	19	POL	414	WLSLDVSAAF	XLXXXXXXXF	
95	19	POL	414	WLSLDVSAAFY	XLXXXXXXXXY	
100	20	ENV	335	WLSLLVPF	XLXXXXXF	
100	20	ENV	335	WLSLLVPFVQW	XLXXXXXXXXW	0.0810
85	17	NUC	26	WLWGMDIDPY	XLXXXXXXXY	
95	19	ENV	237	WMCLRRFIIF	XMXXXXXXXF	
85	17	ENV	359	WMMWYWGPS	XMXXXXXXXXY	
100	20	POL	52	WTHKVGNF	XTXXXXXF	

HBV A01 SUPER MOTIF(With binding Information)

Conservancy	Freq.	Protein	Position	Sequence	String	A*0101
100	20	POL	122	YLPLDKGIKPY	XLXXXXXXXXY	
90	18	NUC	118	YLVSFGVW	XLXXXXXW	
80	16	POL	493	YSHPIILGF	XSXXXXXXF	
85	17	POL	580	YSLNFMGY	XSXXXXXY	
				148		

Table VIII

HBV A02 Super Motif with Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0201	A*0202	A*0203	A*0206	A*6802
85	17	POL	721	AACFARSRSGA	11					
85	17	POL	431	AAMPHLLV	8					
80	16	POL	756	AANWILRGT	9					
95	19	POL	632	AAPFTQCGYPA	11					
95	19	POL	521	AICSVVRRA	9	0.0001				
90	18	NUC	58	AILCWGEL	8					
90	18	NUC	58	AILCWGELM	9					
95	19	POL	642	ALMPLYACI	9	0.5000	0 0340	3 3000	0.0250	0.0470
80	16	ENV	108	AMQWNSTT	8					
75	15	X	102	AMSTTDLEA	9	0.0013				
95	19	POL	516	AQFTSAICSV	10					
95	19	POL	516	AQFTSAICSVV	11					
95	19	POL	690	ATPTGWGL	8					
80	16	POL	690	ATPTGWGLA	9					
75	15	POL	690	ATPTGWGLAI	10					
95	19	POL	397	AVPNLQSL	8					
95	19	POL	397	AVPNLQSLT	9	0.0001				
95	19	POL	397	AVPNLQSLTNL	11					
80	16	POL	755	CAANWILRGT	10					
95	19	X	61	CAFSSAGPCA	10	0.0001				
95	19	X	61	CAFSSAGPCAL	11					
90	18	X	69	CALRFTSA	8					
100	20	ENV	312	CIPIPSSWA	9	0.0010				
80	16	ENV	312	CIPIPSSWAFA	11					
90	18	POL	533	CLAFSYMDDV	10	0 0008				
90	18	POL	533	CLAFSYMDDVV	11					
85	17	NUC	23	CLGWLWGM	8					
85	17	NUC	23	CLGWLWGMDI	10	0.0093				
100	20	ENV	253	CLIFLLVL	8	0.0002				
100	20	ENV	253	CLIFLLVLL	9	0.0006				
95	19	ENV	239	CLRRFIIFL	9	0.0002				
75	15	ENV	239	CLRRFIIFLFI	11	0.0004				
90	18	NUC	107	CLTFGRET	8					
90	18	NUC	107	CLTFGRETV	9	0 0001				
80	16	X	7	CQLDPARDV	9					
80	16	X	7	CQLDPARDVL	10					
85	17	POL	622	CQRIVGLL	8					
85	17	POL	622	CQRIVGLLGFA	11					
95	19	POL	684	CQVFADAT	8					
95	19	POL	684	CQVFADATPT	10					
100	20	ENV	310	CTCIPIPSSWA	11					
95	19	POL	689	DATPTGWGL	9	0 0001				
80	16	POL	689	DATPTGWGLA	10					
75	15	POL	689	DATPTGWGLAI	11					
90	18	NUC	31	DIDPYKEFGA	10					
85	17	NUC	29	DLLDTASA	8					

HBV A02 Super Motif with Binding Information

Conservancy	Freq	Protein	Position	Sequence	AA	A*0201	A*0202	A*0203	A*0206	A*6802
85	17	NUC	29	DLLDTASAL	9	0 0001				
95	19	POL	40	DLNLGNLNV	9	0.0004				
95	19	POL	40	DLNLGNLNVSI	11					
80	16	NUC	32	DTASALYREA	10					
80	16	NUC	32	DTASALYREAL	11					
95	19	X	14	DVLCLRPV	8					
95	19	X	14	DVLCLRPVGA	10	0.0001				
90	18	POL	541	DVVLGAKSV	9	0 0003				
100	20	POL	17	EAGPLEEEL	9	0.0001				
80	16	X	122	ELGEEIRL	8					
90	18	POL	718	ELLAACFA	8					
75	15	NUC	142	ETVLEYLV	8					
95	19	POL	687	FADATPTGWGL	11					
85	17	POL	724	FARSRSGA	8					
80	16	POL	821	FASPLHVA	8					
95	19	POL	396	FAVPNLQSL	9					
95	19	POL	396	FAVPNLQSLT	10	0.0003				
80	16	ENV	243	FIIFLFIL	8	0 0006				
80	16	ENV	243	FIIFLFILL	9	0 0002				
80	16	ENV	243	FIIFLFILLL	10	0.0012				
80	16	ENV	248	FILLLCLI	8	0.0003				
80	16	ENV	248	FILLLCLIFL	10	0 0280				
80	16	ENV	248	FILLLCLIFLL	11	0 0010				
80	16	ENV	246	FLFILLLCL	9	0 0002				
80	16	ENV	246	FLFILLLCLI	10	0.0013				
75	15	ENV	171	FLGPLLVL	8					
75	15	ENV	171	FLGPLLVLQA	10	0 0190				
95	19	POL	513	FLLAQFTSA	9	0 2400				
95	19	POL	513	FLLAQFTSAI	10	0.2100	0.0320	7.0000	0 1100	0 0880
95	19	POL	562	FLLSLGIHL	9	0 6500	0.0010	0.0100	0.1100	0.0035
80	16	ENV	183	FLLTRILT	8					
80	16	ENV	183	FLLTRILTI	9	0 5100	0 0430	8 0000	0.2000	0.0010
95	19	ENV	256	FLLVLLDYQGM	11					
100	20	POL	363	FLVDKNPHNT	10	0 0012				
95	19	POL	656	FTFSPTYKA	9	0.0056	0.0150	0 0031	0 0008	7 3000
95	19	POL	656	FTFSPTYKAFL	11					
95	19	POL	59	FTGLYSST	8					
90	18	POL	59	FTGLYSSTV	9	0.0005				
95	19	POL	635	FTQCGYPA	8					
95	19	POL	635	FTQCGYPAL	9	0 0009				
95	19	POL	635	FTQCGYPALM	10	0 0024				
95	19	POL	518	FTSAICSV	8					
95	19	POL	518	FTSAICSVV	9	0 0090				
95	19	ENV	346	FVGLSPTV	8					
95	19	ENV	346	FVGLSPTVWL	10	0 0008				
90	18	X	132	FVLGGCRHKL	10	0.0030				

HBV A02 Super Motif with Binding Information

Conservancy	Freq	Protein	Position	Sequence	AA	A*0201	A*0202	A*0203	A*0206	A*6802
90	18	X	132	FVLGGCRHKLV	11					
95	19	ENV	342	FVQWFVGL	8					
95	19	ENV	342	FVQWFVGLSPT	11					
90	18	POL	766	FVYVPSAL	8					
90	18	POL	766	FVYVPSALNPA	11					
95	19	X	50	GAHLSLRGL	9	0.0001				
90	18	X	50	GAHLSLRGLPV	11					
85	17	POL	545	GAKSVQHL	8					
85	17	POL	545	GAKSVQHLESL	11					
75	15	POL	567	GIHLNPNKT	9					
90	18	POL	155	GILYKRET	8					
90	18	POL	155	GILYKRETT	9					
85	17	POL	682	GLCQVFADA	9	0.0024				
85	17	POL	682	GLCQVFADAT	10					
95	19	POL	627	GLLGFAAPFT	10	0.0049				
85	17	ENV	62	GLLGWSPQA	9	0.4000	0 0003	0 0350	0 0028	0 0005
95	19	X	57	GLPVCAFSSA	10	0 0008				
95	19	POL	509	GLSPFLLA	8					
95	19	POL	509	GLSPFLLAQFT	11					
100	20	ENV	348	GLSPTVWL	8	0.0036				
75	15	ENV	348	GLSPTVWLSV	10	0.2800				
75	15	ENV	348	GLSPTVWLSVI	11	0.0036				
90	18	ENV	265	GMLPVCPL	8					
90	18	POL	735	GTDNSVVL	8					
75	15	ENV	13	GTNLSVPNPL	10					
80	16	POL	763	GTSFVYVPSA	10					
80	16	POL	763	GTSFVYVPSAL	11					
80	16	POL	507	GVGLSPFL	8					
80	16	POL	507	GVGLSPFLL	9	0 0002				
80	16	POL	507	GVGLSPFLLA	10					
95	19	NUC	123	GVWIRTPPA	9	0.0030				
90	18	NUC	104	HISCLTFGRET	11					
80	16	POL	435	HLLVGSSGL	9	0.0031				
90	18	X	52	HLSLRGLPV	9	0.0014				
90	18	X	52	HLSLRGLPVCA	11					
80	16	POL	491	HLYSHPII	8					
80	16	POL	491	HLYSHPIIL	9	0.2200	0 0003	0.9300	0.0017	0.0530
85	17	POL	715	HTAELLAA	8					
85	17	POL	715	HTAELLAACFA	11					
100	20	NUC	52	HTALRQAI	8					
95	19	NUC	52	HTALRQAIL	9	0.0001				
100	20	POL	149	HTLWKAGI	8					
100	20	POL	149	HTLWKAGIL	9	0.0001				
80	16	ENV	244	IIFLFILL	8	0.0004				
80	16	ENV	244	IIFLFILLL	9	0 0002				
80	16	ENV	244	IIFLFILLLCL	11	0.0002				

HBV A02 Super Motif with Binding Information

Conservancy	Freq	Protein	Position	Sequence	AA		A*0201	A*0202	A*0203	A*0206	A*6802
80	16	POL	497	IILGFRKI	8						
80	16	POL	497	IILGFRKIPM	10						
90	18	NUC	59	ILCWGELM	8						
80	16	POL	498	ILGFRKIPM	9		0.0002				
100	20	ENV	249	ILLLCLIFL	9	*	0.0015				
100	20	ENV	249	ILLLCLIFLL	10	*	0.0190	0.0001	0 0002	0.0013	0 0015
100	20	ENV	249	ILLLCLIFLLV	11		0.0056				
80	16	POL	760	ILRGTSFV	8						
80	16	POL	760	ILRGTSFVYV	10	*	0 0160				
100	20	NUC	139	ILSTLPET	8						
100	20	NUC	139	ILSTLPETT	9		0 0001				
100	20	NUC	139	ILSTLPETTV	10	*	0.0210	0.0085	0.0770	0.0031	0 0067
100	20	NUC	139	ILSTLPETTVV	11						
95	19	ENV	188	ILTIPQSL	8						
90	18	POL	156	ILYKRETT	8						
90	18	POL	625	IVGLLGFA	8						
90	18	POL	625	IVGLLGFAA	9		0.0009				
90	18	POL	153	KAGILYKRET	10						
90	18	POL	153	KAGILYKRETT	11						
80	16	POL	503	KIPMGVGL	8						
85	17	NUC	21	KLCLGWLWGM	10	*	0 0001				
95	19	POL	489	KLHLYSHPI	9	*	0 0690	0.0340	2.7000	0.0059	0 0015
80	16	POL	489	KLHLYSHPII	10						
80	16	POL	489	KLHLYSHPIIL	11						
80	16	POL	610	KLPVNRPI	8						
95	19	POL	653	KQAFTFSPT	9						
95	19	POL	574	KTKRWGYSL	9		0 0001				
85	17	POL	620	KVCQRIVGL	9		0.0003				
85	17	POL	620	KVCQRIVGLL	10		0.0001				
95	19	POL	55	KVGNFTGL	8						
85	17	X	91	KVLHKRTL	8						
85	17	X	91	KVLHKRTLGL	10		0 0004				
90	18	POL	534	LAFSYMDDV	9		0 0002				
90	18	POL	534	LAFSYMDDVV	10		0.0003				
90	18	POL	534	LAFSYMDDVVL	11						
95	19	POL	515	LAQFTSAI	8						
95	19	POL	515	LAQFTSAICSV	11						
100	20	ENV	254	LIFLLVLL	8		0.0025				
95	19	POL	514	LLAQFTSA	8						
95	19	POL	514	LLAQFTSAI	9	*	0 1000	0.2700	3.7000	0.0026	0 7900
100	20	ENV	251	LLCLIFLL	8		0 0004				
100	20	ENV	251	LLCLIFLLV	9	*	0.0048				
100	20	ENV	251	LLCLIFLLVL	10		0.0075				
100	20	ENV	251	LLCLIFLLVLL	11		0 0013				
85	17	NUC	30	LLDTASAL	8						
95	19	ENV	260	LLDYQGML	8		0.0004				

HBV A02 Super Motif with Binding Information

Conservancy	Freq	Protein	Position	Sequence	AA		A*0201	A*0202	A*0203	A*0206	A*6802
90	18	ENV	260	LLDYQGMLPV	10	*	0.0980	0.0001	0.0200	0.0067	0.0009
80	16	POL	752	LLGCAANWI	9		0 0011				
80	16	POL	752	LLGCAANWIL	10	*	0.0140				
95	19	POL	628	LLGFAAPFT	9		0 0008				
85	17	ENV	63	LLGWSPQA	8						
75	15	ENV	63	LLGWSPQAQGI	11						
100	20	ENV	250	LLLCLIFL	8		0 0006				
100	20	ENV	250	LLLCLIFLL	9	*	0 0065				
100	20	ENV	250	LLLCLIFLLV	10	*	0 0036				
100	20	ENV	250	LLLCLIFLLVL	11		0 0005				
100	20	ENV	378	LLPIFFCL	8		0 0055				
100	20	ENV	378	LLPIFFCLWV	10	*	0.0320	0 0008	0.0150	0 0008	0 0005
95	19	POL	563	LLSLGIHL	8						
90	18	POL	407	LLSSNLSWL	9	*	0.0110	0 0780	3 9000	0.0027	0.0100
90	18	POL	407	LLSSNLSWLSL	11						
80	16	ENV	184	LLTRILTI	8		0 0026				
80	16	POL	436	LLVGSSGL	8						
95	19	ENV	257	LLVLLDYQGM	10		0.0050				
95	19	ENV	257	LLVLLDYQGML	11						
90	18	ENV	175	LLVLQAGFFL	10	*	0 0310	0 0037	0 0045	0.0015	0.0110
90	18	ENV	175	LLVLQAGFFLL	11		0 0074				
95	19	ENV	338	LLVPFVQWFV	10	*	0.6700	0 3800	1 7000	0.2900	0 1400
90	18	NUC	100	LLWFHISCL	9	*	0.0130	0 0002	0.0420	0.0031	0.0098
85	17	NUC	100	LLWFHISCLT	10						
95	19	POL	643	LMPLYACI	8						
95	19	ENV	178	LQAGFFLL	8						
95	19	ENV	178	LQAGFFLLT	9						
80	16	ENV	178	LQAGFFLLTRI	11						
100	20	POL	401	LQSLTNLL	8						
95	19	NUC	108	LTFGRETV	8						
75	15	NUC	137	LTFGRETVL	9						
90	18	POL	404	LTNLLSSNL	9						
80	16	ENV	185	LTRILTIPQSL	11						
85	17	POL	99	LTVNEKRRL	9						
100	20	POL	364	LVDKNPHNT	9		0 0001				
95	19	ENV	258	LVLLDYQGM	9		0 0001				
95	19	ENV	258	LVLLDYQGML	10		0 0001				
90	18	ENV	176	LVLQAGFFL	9		0.0096				
90	18	ENV	176	LVLQAGFFLL	10	*	0.0022				
90	18	ENV	176	LVLQAGFFLLT	11						
95	19	ENV	339	LVPFVQWFV	9	*	0.0420	0 0150	0 0048	0.0079	2 8000
95	19	ENV	339	LVPFVQWFVGL	11						
90	18	NUC	119	LVSFGVWI	8		0.0004				
90	18	NUC	119	LVSFGVWIRT	10						
85	17	ENV	360	MMWYWGPSL	9	*	0 6400				
75	15	NUC	1	MQLFHLCL	8						

HBV A02 Super Motif with Binding Information

Conservancy	Freq	Protein	Position	Sequence	AA	A*0201	A*0202	A*0203	A*0206	A*6802
100	20	NUC	136	NAPILSTL	8					
100	20	NUC	136	NAPILSTLPET	11					
95	19	POL	42	NLGNLNVSI	9	0 0047				
90	18	POL	406	NLLSSNLSWL	10	0.0016				
95	19	POL	45	NLNVSIPWT	9	0 0005				
100	20	POL	400	NLQSLTNL	8					
100	20	POL	400	NLQSLTNLL	9	0.0047				
75	15	ENV	15	NLSVPNPL	8					
90	18	POL	411	NLSWLSLDV	9	0.0650	0 0051	0.6400	0 0016	0.0990
90	18	POL	411	NLSWLSLDVSA	11					
100	20	POL	47	NVSIPWTHKV	10	0 0001				
100	20	POL	430	PAAMPHLL	8					
85	17	POL	430	PAAMPHLLV	9					
90	18	POL	775	PADDPSRGRL	10					
90	18	ENV	131	PAGGSSSGT	9					
90	18	ENV	131	PAGGSSSGTV	10					
95	19	POL	641	PALMPLYA	8					
95	19	POL	641	PALMPLYACI	10	0 0001				
75	15	X	145	PAPCNFFT	8					
75	15	X	145	PAPCNFFTSA	10					
80	16	X	11	PARDVLCL	8					
75	15	X	11	PARDVLCLRPV	11					
90	18	POL	355	PARVTGGV	8					
90	18	POL	355	PARVTGGVFL	10					
90	18	POL	355	PARVTGGVFLV	11					
95	19	NUC	130	PAYRPPNA	8					
95	19	NUC	130	PAYRPPNAPI	10	0.0001				
95	19	NUC	130	PAYRPPNAPIL	11					
85	17	POL	616	PIDWKVCQRI	10	0.0001				
85	17	POL	616	PIDWKVCQRIV	11					
100	20	ENV	380	PIFFCLWV	8					
100	20	ENV	380	PIFFCLWVYI	10	0 0004				
85	17	POL	713	PIHTAELL	8					
85	17	POL	713	PIHTAELLA	9					
85	17	POL	713	PIHTAELLAA	10					
80	16	POL	496	PIILGFRKI	9	0 0001				
80	16	POL	496	PIILGFRKIPM	11					
100	20	NUC	138	PILSTLPET	9	0 0001				
100	20	NUC	138	PILSTLPETT	10	0 0001				
100	20	NUC	138	PILSTLPETTV	11	0 0001				
80	16	ENV	314	PIPSSWAFA	9					
95	19	POL	20	PLEEELPRL	9	0 0003				
90	18	POL	20	PLEEELPRLA	10	0.0001				
95	19	ENV	10	PLGFFPDHQL	10	0 0002				
100	20	POL	427	PLHPAAMPHL	10	0.0001				
100	20	POL	427	PLHPAAMPHLL	11					

HBV A02 Super Motif with Binding Information

Conservancy	Freq	Protein	Position	Sequence	AA	A*0201	A*0202	A*0203	A*0206	A*6802
100	20	ENV	377	PLLPIFFCL	9	0.0650	0.0001	0.0018	0 0011	0 0047
100	20	ENV	377	PLLPIFFCLWV	11					
90	18	ENV	174	PLLVLQAGFFL	11	0.0008				
80	16	POL	711	PLPIHTAEL	9	0.0004				
80	16	POL	711	PLPIHTAELL	10	0.0001				
80	16	POL	711	PLPIHTAELLA	11					
75	15	POL	2	PLSYQHFRKL	10	0 0001				
75	15	POL	2	PLSYQHFRKLL	11					
85	17	POL	98	PLTVNEKRRL	10	0 0001				
80	16	POL	505	PMGVGLSPFL	10	0 0001				
80	16	POL	505	PMGVGLSPFLL	11					
95	19	ENV	106	PQAMQWNST	9					
80	16	ENV	106	PQAMQWNSTT	10					
90	18	ENV	192	PQSLDSWWT	9					
90	18	ENV	192	PQSLDSWWTSL	11					
75	15	POL	692	PTGWGLAI	8					
80	16	ENV	219	PTSNHSPT	8					
85	17	POL	797	PTTGRTSL	8					
85	17	POL	797	PTTGRTSLYA	10					
80	16	NUC	15	PTVQASKL	8					
80	16	NUC	15	PTVQASKLCL	10					
75	15	ENV	351	PTVWLSVI	8					
75	15	ENV	351	PTVWLSVIWM	10					
95	19	X	59	PVCAFSSA	8					
85	17	POL	612	PVNRPIDWKV	10	0 0002				
95	19	POL	654	QAFTFSPT	8					
95	19	POL	654	QAFTFSPTYKA	11					
95	19	ENV	179	QAGFFLLT	8					
80	16	ENV	179	QAGFFLLTRI	10					
80	16	ENV	179	QAGFFLLTRIL	11					
90	18	NUC	57	QAILCWGEL	9					
90	18	NUC	57	QAILCWGELM	10					
95	19	ENV	107	QAMQWNST	8					
80	16	ENV	107	QAMQWNSTT	9					
80	16	NUC	18	QASKLCLGWL	10					
80	16	X	8	QLDPARDV	8	0 0001				
80	16	X	8	QLDPARDVL	9	0.0001				
80	16	X	8	QLDPARDVLCL	11	0.0001				
90	18	NUC	99	QLLWFHISCL	10	0.0060				
85	17	NUC	99	QLLWFHISCLT	11					
95	19	POL	685	QVFADATPT	9	0 0001				
95	19	POL	528	RAFPHCLA	8					
80	16	ENV	187	RILTIPQSL	9	0.0010				
90	18	POL	624	RIVGLLGFA	9					
90	18	POL	624	RIVGLLGFAA	10					
75	15	POL	106	RLKLIMPA	8					

HBV A02 Super Motif with Binding Information

Conservancy	Freq	Protein	Position	Sequence	AA	A*0201	A*0202	A*0203	A*0206	A*6802
90	18	NUC	56	RQAILCWGEL	10					
90	18	NUC	56	RQAILCWGELM	11					
90	18	NUC	98	RQLLWFHI	8					
90	18	NUC	98	RQLLWFHISCL	11					
85	17	ENV	88	RQSGRQPT	8					
90	18	POL	353	RTPARVTGGV	10					
95	19	NUC	127	RTPPAYRPPNA	11					
95	19	POL	36	RVAEDLNL	8					
90	18	POL	36	RVAEDLNLGNL	11					
80	16	POL	818	RVHFASPL	8					
75	15	POL	818	RVHFASPLHV	10	0.0001				
75	15	POL	818	RVHFASPLHVA	11					
100	20	POL	357	RVTGGVFL	8					
100	20	POL	357	RVTGGVFLV	9	0.0041				
90	18	X	65	SAGPCALRFT	10					
95	19	POL	520	SAICSVVRRA	10	0 0001				
90	18	NUC	35	SALYREAL	8					
100	20	POL	49	SIPWTHKV	8					
95	19	ENV	194	SLDSWWTSL	9	0 0023				
75	15	POL	565	SLGIHLNPNKT	11					
95	19	ENV	337	SLLVPFVQWFV	11					
75	15	POL	581	SLNFMGYV	8					
75	15	POL	581	SLNFMGYVI	9	0.0038				
95	19	X	54	SLRGLPVCA	9	0 0007				
90	18	POL	403	SLTNLLSSNL	10	0.0014				
75	15	ENV	216	SQSPTSNHSPT	11					
75	15	ENV	280	STGPCKTCT	9					
100	20	NUC	141	STLPETTV	8					
100	20	NUC	141	STLPETTVV	9	0.0019				
80	16	ENV	85	STNRQSGRQPT	11					
85	17	POL	548	SVQHLESL	8					
80	16	ENV	330	SVRFSWLSL	9	0 0001				
80	16	ENV	330	SVRFSWLSLL	10	0.0004				
80	16	ENV	330	SVRFSWLSLLV	11					
90	18	POL	739	SVVLSRKYT	9					
95	19	POL	524	SVVRRAFPHCL	11					
85	17	POL	716	TAELLAACFA	10					
95	19	NUC	53	TALRQAIL	8					
80	16	NUC	33	TASALYREA	9					
80	16	NUC	33	TASALYREAL	10					
90	18	ENV	190	TIPQSLDSWWT	11					
100	20	NUC	142	TLPETTVV	8					
100	20	POL	150	TLWKAGIL	8					
95	19	POL	636	TQCGYPAL	8					
95	19	POL	636	TQCGYPALM	9					
95	19	POL	636	TQCGYPALMPL	11					

HBV A02 Super Motif with Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA		A*0201	A*0202	A*0203	A*0206	A*6802
85	17	POL	798	TTGRTSLYA	9						
75	15	ENV	278	TTSTGPCKT	9						
75	15	ENV	278	TTSTGPCKTCT	11						
85	17	POL	100	TVNEKRRL	8						
80	16	NUC	16	TVQASKLCL	9		0.0002				
75	15	ENV	352	TVWLSVIWM	9		0.0002				
95	19	POL	37	VAEDLNLGNL	10		0.0001				
95	19	X	15	VLCLRPVGA	9		0.0014				
85	17	POL	543	VLGAKSVQHL	10		0.0001				
90	18	X	133	VLGGCRHKL	9		0.0009				
90	18	X	133	VLGGCRHKLV	10		0.0001				
85	17	X	92	VLHKRTLGL	9		0 0012				
95	19	ENV	259	VLLDYQGM	8						
95	19	ENV	259	VLLDYQGML	9	*	0 0440	0 0001	0 0210	0 0009	0 0002
90	18	ENV	259	VLLDYQGMLPV	11	*	0 5800	0 2200	4.9000	0 3400	0 0170
95	19	ENV	177	VLQAGFFL	8		0 0019				
95	19	ENV	177	VLQAGFFLL	9	*	0 0660				
95	19	ENV	177	VLQAGFFLLT	10		0.0011				
80	16	NUC	17	VQASKLCL	8						
80	16	NUC	17	VQASKLCLGWL	11						
95	19	ENV	343	VQWFVGLSPT	10						
95	19	ENV	343	VQWFVGLSPTV	11						
100	20	POL	358	VTGGVFLV	8						
90	18	POL	542	VVLGAKSV	8						
80	16	POL	542	VVLGAKSVQHL	11						
90	18	POL	740	VVLSRKYT	8						
95	19	POL	525	VVRRAFPHCL	10		0.0003				
95	19	POL	525	VVRRAFPHCLA	11						
80	16	POL	759	WILRGTSFV	9	*	0 0270				
80	16	POL	759	WILRGTSFVYV	11						
80	16	POL	751	WLLGCAANWI	10		0.0053				
80	16	POL	751	WLLGCAANWIL	11						
100	20	POL	414	WLSLDVSA	8						
95	19	POL	414	WLSLDVSAA	9		0 0059				
100	20	ENV	335	WLSLLVPFV	9	*	1.1000	0 0380	7.2000	0.3600	0 0310
95	19	ENV	237	WMCLRRFI	8						
95	19	ENV	237	WMCLRRFII	9	*	0.0005				
95	19	ENV	237	WMCLRRFIIFL	11		0 0019				
85	17	ENV	359	WMMWYWGPSL	10	*	0 0009				
100	20	POL	52	WTHKVGNFT	9		0 0001				
95	19	POL	52	WTHKVGNFTGL	11						
100	20	POL	147	YLHTLWKA	8						
100	20	POL	147	YLHTLWKAGI	10	*	0 0160	0 0005	0.5600	0 0010	0 0320
100	20	POL	147	YLHTLWKAGIL	11						
100	20	POL	122	YLPLDKGI	8						
90	18	NUC	118	YLVSFGVWI	9	*	0.3800				

HBV A02 Super Motif with Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA		A*0201	A*0202	A*0203	A*0206	A*6802
90	18	NUC	118	YLVSFGVWIRT	11						
90	18	POL	538	YMDDVVLGA	9		0.0250	0 0001	0 0024	0.0001	0 0002
90	18	ENV	263	YQGMLPVCPL	10						
75	15	POL	5	YQHFRKLL	8						
75	15	POL	5	YQHFRKLLL	9						
75	15	POL	5	YQHFRKLLLL	10						
85	17	POL	746	YTSFPWLL	8						
75	15	POL	746	YTSFPWLLGCA	11						
90	18	POL	768	YVPSALNPA	9		0.0039				
			423		3	45					

TABLE IX

HBV A03 SUPER MOTIF (With binding information)

Conservancy	Frequency	Protein	Position	Sequence	P2	C-term	AA	A*0301	A*1101	A*3101	A*3301	A*6801
85	17	POL	721	AACFARSR	A	R	8	0.0004	0.0003	0.0056	0.0035	0.0014
95	19	POL	521	AICSVVRR	I	R	8	-0.0002	0.0003	0.0014	-0.0009	0.0006
90	18	POL	772	ALNPADDPSR	L	R	10	0.0003	0.0001			
85	17	X	70	ALRFTSAR	L	R	8	0.0047	0.0009	0.0450	0.0230	0.0004
80	16	POL	822	ASPLHVAWR	S	R	9					
75	15	ENV	84	ASTNRQSGR	S	R	9	0.0009	0.0002	0.0088	0.0008	0.0001
80	16	POL	755	CAANWILR	A	R	8					
85	17	X	69	CALRFTSAR	A	R	9	0.0034	0.0230	1.5000	8.0000	0.7300
90	18	X	17	CLRPVGAESR	L	R	10	0.0011	0.0001			
100	20	NUC	48	CSPHHTALR	S	R	9	0.0029	0.0001	0.0520	0.0250	0.0440
85	17	NUC	29	DLLDTASALYR	L	R	11	0.0042	-0.0003	-0.0012	3.7000	0.0410
85	17	NUC	32	DTASALYR	T	R	8	0.0004	-0.0002	-0.0009	0.0018	0.0009
95	19	POL	17	EAGPLEEELPR	A	R	11	-0.0009	-0.0003	-0.0012	0.0015	0.0110
90	18	POL	718	ELLAACFAR	L	R	9	0.0002	0.0004			
85	17	POL	718	ELLAACFARSR	L	R	11	0.0062	0.0016	0.0200	0.2000	0.1600
95	19	NUC	174	ETTVVRRR	T	R	8	0.0003	-0.0002	-0.0009	0.1400	0.0027
80	16	NUC	174	ETTVVRRRGR	T	R	10	0.0003	0.0001			
80	16	POL	821	FASPLHVAWR	A	R	10					
90	18	X	83	FSSAGPCALR	S	R	10					
95	19	POL	856	FTFSPTYK	T	K	8	0.0100	0.0100	0.0023	0.2100	0.0590
95	19	POL	518	FTSAICSVVR	T	R	10	0.0003	0.0003			
95	19	POL	518	FTSAICSVVRR	T	R	11	0.0065	0.0092	0.0170	0.0350	1.5000
90	18	X	132	FVLGGCRHK	V	K	9	0.0430	0.0090			
75	15	POL	567	GIHLNPNK	I	K	8					
75	15	POL	567	GIHLNPNKTK	I	K	10	0.0025	0.0011	0.0009	0.0009	0.0003
75	15	POL	567	GIHLNPNKTKR	I	R	11					
85	17	NUC	29	GMDIDPYK	M	K	8	0.0006	0.0004	-0.0009	-0.0009	0.0001
90	18	POL	735	GTDNSVVLSR	T	R	10	0.0010	0.0420	0.0030	0.0019	0.0008
90	18	POL	735	GTDNSVVLSRK	T	K	11	0.0140	0.5600	-0.0002	-0.0006	0.0001
95	19	NUC	123	GVWIRTPPAYR	V	R	11	0.1900	0.1700	6.8000	0.7300	0.6600
90	18	NUC	104	HISCLTFGR	I	R	9	0.0160	0.0065			
75	15	POL	568	HLNPNKTK	L	K	8					
75	15	POL	568	HLNPNKTKR	L	R	9	0.0025	0.0001			
100	20	POL	149	HTLWKAGILYK	T	K	11	0.5400	0.4400	0.0370	0.0720	0.1900
90	18	NUC	105	ISCLTFGR	S	R	8	0.0004	0.0002	0.0017	-0.0009	0.0017
100	20	POL	153	KAGILYKR	A	R	8	0.0002	-0.0002	0.0015	-0.0009	0.0001
80	16	POL	610	KLPVNRPIDWK	L	K	11					
75	15	X	130	KVFVLGGCR	V	R	9	0.0420	0.0820	0.6000	0.0710	0.0030
85	17	POL	720	LAACFARSR	A	R	9	0.0058	0.0065			
90	18	POL	719	LLAACFAR	L	R	8	0.0024	0.0003	0.0015	0.0028	0.0064
85	17	POL	719	LLAACFARSR	L	R	10					
85	17	NUC	30	LLDTASALYR	L	R	10	0.0050	0.0002			
80	16	POL	752	LLGCAANWILR	L	R	11					
75	15	POL	564	LSLGIHLNPNK	S	K	11					
95	19	NUC	169	LSTLPETTVVR	S	R	11	-0.0009	0.0008	-0.0012	-0.0023	0.0078
75	15	POL	3	LSYQHFRK	S	K	8					
85	17	POL	98	LTVNEKRR	T	R	8	-0.0002	-0.0002	-0.0009	-0.0009	0.0001
90	18	NUC	118	LVSFGVWIR	V	R	9	0.0028	0.0120			
100	20	POL	377	LVVDFSQFSR	V	R	10	0.0016	0.3600	0.0260	0.2300	0.4900
75	15	X	103	MSTTDLEAYFK	S	K	11					
90	18	NUC	75	NLEDPASR	L	R	8	-0.0002	-0.0002	-0.0009	-0.0009	0.0001

HBV A03 SUPER MOTIF (With binding Information)

Conservancy	Frequency	Protein	Position	Sequence	P2	C-term	AA	A*0301	A*1101	A*3101	A*3301	A*6801
95	19	POL	45	NLNVSIPWTHK	L	K	11	-0.0009	0.0005	-0.0012	-0.0023	0.0019
90	18	POL	738	NSVVLSRK	S	K	8	0.0006	0.0010	-0.0009	-0.0009	0.0007
100	20	POL	47	NVSIPWTHK	V	K	9	0.0820	0.0570	0.0002	0.0100	0.0320
90	18	POL	775	PADDPSRGR	A	R	9	0.0008	0.0002	0.0004	0.0015	0.0002
80	16	X	11	PARDVLCLR	A	R	9	0.0002	0.0002	0.0100	0.0180	0.0002
75	15	ENV	83	PASTNRQSGR	A	R	10					
90	18	POL	616	PIDWKVCQR	I	R	9	0.0002	0.0005			
80	16	POL	496	PIILGFRK	I	K	8					
95	19	POL	20	PLEEELPR	L	R	8	0.0002	-0.0002	-0.0009	-0.0009	0.0001
100	20	POL	2	PLSYQHFR	L	R	8	-0.0002	-0.0002	-0.0009	-0.0009	0.0001
75	15	POL	2	PLSYQHFRK	L	K	9	0.0011	0.0031	0.0006	0.0008	0.0002
85	17	POL	98	PLTVNEKR	L	R	8	0.0002	-0.0002	-0.0009	-0.0009	0.0001
85	17	POL	98	PLTVNEKRR	L	R	9	0.0008	0.0005	0.0004	0.0027	0.0002
90	18	X	20	PVGAESRGR	V	R	9	0.0002	0.0005	0.0004	0.0043	0.0002
85	17	POL	612	PVNRPIDWK	V	K	9	0.0310	0.1400	0.0002	0.0006	0.0009
95	19	POL	654	QAFTFSPTYK	A	K	10	0.0450	0.5400	0.0010	0.0057	1.2000
80	16	ENV	179	QAGFFLLTR	A	R	9					
75	15	NUC	169	QSPRRRRSQSR	S	R	11					
80	16	POL	189	QSSGILSR	S	R	8					
75	15	POL	106	RLKLIMPAR	L	R	9	0.0950	0.0002	3.1000	0.0490	0.0002
75	15	X	128	RLKVFVLGGCR	L	R	11					
95	19	POL	376	RLVVDFSQFSR	L	R	11	0.2800	3.8000	2.6000	1.2000	6.1000
95	19	NUC	183	RSPRRRTPSPR	S	R	11	-0.0007	-0.0003	0.0190	-0.0023	0.0003
75	15	NUC	167	RSQSPRRR	S	R	8					
75	15	NUC	167	RSQSPRRRR	S	R	9					
95	19	NUC	168	RTPSPRRR	T	R	8	-0.0002	-0.0002	0.0033	0.0014	0.0002
95	19	NUC	168	RTPSPRRRR	T	R	9	0.0054	0.0005	0.2000	0.0016	0.0003
100	20	POL	357	RVTGGVFLVDK	V	K	11	0.0190	0.0290	-0.0002	-0.0003	0.0001
90	18	X	65	SAGPCALR	A	R	8	-0.0002	0.0020	0.0029	0.0024	0.0360
95	19	POL	520	SAICSVVR	A	R	8	-0.0002	0.0071	0.0280	0.0081	0.0690
95	19	POL	520	SAICSVVRR	A	R	9	0.0058	0.2100	0.0150	0.0650	0.3800
90	18	POL	771	SALNPADDPSR	A	R	11	-0.0004	-0.0003	-0.0012	-0.0023	0.0003
75	15	POL	585	SLGIHLNPNK	L	K	10					
90	18	X	64	SSAGPCALR	S	R	9	0.0080	0.1400	0.3300	0.1600	0.7500
95	19	NUC	170	STLPETTVVR	T	R	10	0.0007	0.0600	0.0080	0.0240	0.0250
95	19	NUC	170	STLPETTVVRR	T	R	11	0.0150	1.4000	0.1000	0.1600	0.3100
80	16	ENV	85	STNRQSGR	T	R	8					
75	15	X	104	STTDLEAYFK	T	K	10	0.0066	2.7000			
85	17	POL	716	TAELLAACFAR	A	R	11	0.0008	0.0023	0.0066	0.1600	0.0590
95	19	NUC	171	TLPETTVVR	L	R	9	0.0008	0.0002	0.0009	0.0024	0.0180
95	19	NUC	171	TLPETTVVRR	L	R	10	0.0007	0.0230	0.0006	0.0120	0.0440
95	19	NUC	171	TLPETTVVRRR	L	R	11	0.0005	0.0160	0.0061	0.0710	0.6400
100	20	POL	150	TLWKAGILYK	L	K	10	5.3000	0.3600	0.0051	0.0010	0.0130
100	20	POL	150	TLWKAGILYKR	L	R	11	0.0082	0.0095	0.1000	0.1100	0.0840
95	19	POL	519	TSAICSVVR	S	R	9	0.0005	0.0008	0.0600	0.0200	0.0820
95	19	POL	519	TSAICSVVRR	S	R	10	0.0018	0.0006	0.0030	0.0066	0.0048
75	15	X	105	TTDLEAYFK	T	K	9	0.0006	0.9200	0.0006	0.0012	0.0170
75	15	ENV	278	TTSTGPCK	T	K	8					
80	16	NUC	175	TTVVRRAGR	T	R	9	0.0008	0.0005	0.2500	0.1400	0.0095
80	16	NUC	176	TVVRRRGR	V	R	8	0.0003	0.0001			
80	16	NUC	176	TVVRRRGRSPR	V	R	11					

HBV A03 SUPER MOTIF (With binding information)

Conservancy	Frequency	Protein	Position	Sequence	P2	C-term	AA	A*0301	A*1101	A*3101	A*3301	A*6801
90	18	X	133	VLGGCRHK	L	K	8	0.0150	0.0002	-0.0005	-0.0009	0.0001
80	16	ENV	177	VLQAGFFLLTR	L	R	11	0.0040	0.0290	0.0750	0.0270	0.0360
90	18	NUC	120	VSFGVWIR	S	R	8	0.0130	0.0170	0.0031	0.0013	0.0004
100	20	POL	48	VSIPWTHK	S	K	8	0.0390	0.0920	0.0002	0.0006	0.0022
100	20	POL	358	VTGGVFLVDK	T	K	10	0.0015	0.0750	0.0013	0.0170	0.0330
100	20	POL	378	VVDFSQFSR	V	R	9	0.0027	0.0001			
80	16	NUC	177	VVRRAGRSPR	V	R	10					
80	16	NUC	177	VVRRAGRSPRR	V	R	11	0.0008	0.0005			
95	19	NUC	125	WIRTPPAYR	I	R	9	-0.0002	0.0005	0.0020	0.0052	0.0001
90	18	POL	314	WLQFRNSK	L	K	8	0.0030	0.0013	-0.0003	0.0039	0.0490
85	17	NUC	26	WLWGMDIDPYK	L	K	11	0.0001	0.0001	0.0006	0.0006	0.0002
100	20	POL	122	YLPLDKGIK	L	K	9	0.0005	0.0002			
90	18	NUC	118	YLVSFGVWIR	L	R	10	0.0330	0.0043	0.0002	0.0006	0.0001
90	18	POL	538	YMDDVVLGAK	M	K	10					
80	16	POL	493	YSHPIILGFR	S	R	10					
80	16	POL	493	YSHPIILGFRK	S	K	11					

Table X

HBV A24 SUPER MOTIF (With binding information)

Conservancy	Freq	Protein	Position	Sequence	String	A*2401
95	19	POL	529	AFPHCLAF	XFXXXXXF	
95	19	POL	529	AFPHCLAFSY	XFXXXXXXXY	
95	19	POL	529	AFPHCLAFSYM	XFXXXXXXXXM	0.0012
95	19	X	82	AFSSAGPCAL	XFXXXXXXL	0.0009
90	18	POL	535	AFSYMDDVVL	XFXXXXXXL	
95	19	POL	655	AFTFSPTY	XFXXXXXY	
95	19	POL	655	AFTFSPTYKAF	XFXXXXXXXXF	
95	19	POL	521	AICSVVRRAF	XIXXXXXXXF	
90	18	NUC	58	AILCWGEL	XIXXXXXL	
90	18	NUC	58	AILCWGELM	XIXXXXXXM	
95	19	POL	642	ALMPLYACI	XLXXXXXXI	
95	19	NUC	54	ALRQAILCW	XLXXXXXXW	
80	16	ENV	108	AMQWNSTTF	XMXXXXXXF	
95	19	POL	690	ATPTGWGL	XTXXXXXL	
75	15	POL	690	ATPTGWGLAI	XTXXXXXXXI	
95	19	POL	397	AVPNLQSL	XVXXXXXL	
95	19	POL	397	AVPNLQSLTNL	XVXXXXXXXXL	0.0260
100	20	NUC	131	AYRPPNAPI	XYXXXXXXI	0.0220
100	20	NUC	131	AYRPPNAPIL	XYXXXXXXXL	
75	15	POL	607	CFRKLPVNRPI	XFXXXXXXXXI	
100	20	ENV	312	CIPIPSSW	XIXXXXXW	
100	20	ENV	312	CIPIPSSWAF	XIXXXXXXXF	
85	17	NUC	23	CLGWLWGM	XLXXXXXM	
85	17	NUC	23	CLGWLWGMDI	XLXXXXXXXI	
100	20	ENV	253	CLIFLLVL	XLXXXXXL	
100	20	ENV	253	CLIFLLVLL	XLXXXXXXL	
95	19	ENV	253	CLIFLLVLLDY	XLXXXXXXXXY	
95	19	ENV	239	CLRRFIIF	XLXXXXXF	
95	19	ENV	239	CLRRFIIFL	XLXXXXXXL	
75	15	ENV	239	CLRRFIIFLF	XLXXXXXXXF	
75	15	ENV	239	CLRRFIIFLFI	XLXXXXXXXXI	
100	20	ENV	310	CTCIPIPSSW	XTXXXXXXXW	
90	18	NUC	31	DIDPYKEF	XIXXXXXF	
85	17	NUC	29	DLLDTASAL	XLXXXXXL	
85	17	NUC	29	DLLDTASALY	XLXXXXXXXY	
95	19	POL	40	DLNLGNLNVSI	XLXXXXXXXXI	
80	16	NUC	32	DTASALYREAL	XTXXXXXXXXL	
85	17	POL	618	DWKVCQRI	XWXXXXXI	
85	17	POL	618	DWKVCQRIVGL	XWXXXXXXXXL	0.0002
90	18	ENV	262	DYCQGMLPVCPL	XYXXXXXXXXL	
80	16	X	122	ELGEEIRL	XLXXXXXL	
95	19	NUC	43	ELLSFLPSDF	XLXXXXXXXF	
95	19	NUC	43	ELLSFLPSDFF	XLXXXXXXXXF	
90	18	NUC	117	EYLVSFGVW	XYXXXXXXW	0.0340
90	18	NUC	117	EYLVSFGVWI	XYXXXXXXXI	
100	20	ENV	382	FFCLWVYI	XFXXXXXI	
80	16	ENV	182	FFLLTRIL	XFXXXXXXL	
80	16	ENV	182	FFLLTRILTI	XFXXXXXXXXI	
85	17	ENV	13	FFPDHQLDPAF	XFXXXXXXXXF	
80	16	ENV	243	FIIFLFIL	XIXXXXXL	
80	16	ENV	243	FIIFLFILL	XIXXXXXXL	
80	16	ENV	243	FIIFLFILLL	XIXXXXXXXL	
80	16	ENV	248	FILLLCLI	XIXXXXXI	
80	16	ENV	248	FILLLCLIF	XIXXXXXXXF	
80	16	ENV	248	FILLLCLIFL	XIXXXXXXXL	
80	16	ENV	248	FILLLCLIFLL	XIXXXXXXXXL	
80	16	ENV	246	FLFILLLCL	XLXXXXXXL	
80	16	ENV	246	FLFILLLCLI	XLXXXXXXXI	
80	16	ENV	246	FLFILLLCLIF	XLXXXXXXXXF	
75	15	ENV	171	FLGPLLVL	XLXXXXXL	
95	19	POL	513	FLLAQFTSAI	XLXXXXXXXI	
95	19	POL	562	FLLSLGIHL	XLXXXXXXL	
80	16	ENV	183	FLLTRILTI	XLXXXXXXI	
95	19	ENV	258	FLLVLLDY	XLXXXXXY	
95	19	ENV	256	FLLVLLDYQGM	XLXXXXXXXXM	
95	19	POL	656	FTFSPTYKAF	XTXXXXXXXF	
95	19	POL	656	FTFSPTYKAFL	XTXXXXXXXXL	
95	19	POL	635	FTQCGYPAL	XTXXXXXXL	
95	19	POL	635	FTQCGYPALM	XTXXXXXXXM	
95	19	ENV	346	FVGLSPTVW	XVXXXXXXW	
95	19	ENV	346	FVGLSPTVWL	XVXXXXXXXL	
90	18	X	132	FVLGGCRHKL	XVXXXXXXXL	
95	19	ENV	342	FVQWFVGL	XVXXXXXL	
90	18	POL	766	FVYVPSAL	XVXXXXXL	
95	19	POL	630	GFAAPFTQCGY	XFXXXXXXXXY	
80	16	ENV	181	GFFLLTRI	XFXXXXXXI	
80	16	ENV	181	GFFLLTRIL	XFXXXXXXL	
80	16	ENV	181	GFFLLTRILTI	XFXXXXXXXXI	

HBV A24 SUPER MOTIF (With binding information)

Conservancy	Freq	Protein	Position	Sequence	String	A*2401
95	19	ENV	12	GFFPDHQL	XFXXXXXL	
75	15	ENV	170	GFLGPLLVL	XFXXXXXXL	
80	16	POL	500	GFRKIPMGVGL	XFXXXXXXXXL	
95	19	POL	627	GLLGFAAPF	XLXXXXXXF	
95	19	POL	509	GLSPFLLAQF	XLXXXXXXXF	
100	20	ENV	348	GLSPTVWL	XLXXXXXL	
75	15	ENV	348	GLSPTVWLSVI	XLXXXXXXXXI	
85	17	NUC	29	GMDIDPYKEF	XMXXXXXXXF	
90	18	ENV	265	GMLPVCPL	XMXXXXXL	
90	18	POL	735	GTDNSVVL	XTXXXXXL	
75	15	ENV	13	GTNLSVPNPL	XTXXXXXXXL	
80	16	POL	763	GTSFVYVPSAL	XTXXXXXXXXL	
80	16	POL	507	GVGLSPFL	XVXXXXXL	
80	16	POL	507	GVGLSPFLL	XVXXXXXXL	
95	19	NUC	123	GVWIRTPPAY	XVXXXXXXXY	
85	17	NUC	25	GWLWGMDI	XWXXXXXXI	
85	17	NUC	25	GWLWGMDIDPY	XWXXXXXXXXY	0.0024
85	17	ENV	65	GWSPQAQGI	XWXXXXXXI	0.0003
85	17	ENV	65	GWSPQAQGIL	XWXXXXXXXL	
95	19	POL	639	GYPALMPL	XYXXXXXL	0.0490
95	19	POL	639	GYPALMPLY	XYXXXXXXY	0.0110
95	19	ENV	234	GYRWMCLRRF	XYXXXXXXXF	
95	19	ENV	234	GYRWMCLRRFI	XYXXXXXXXXI	0.0002
95	19	POL	579	GYSLNFMGY	XYXXXXXXXY	
85	17	POL	579	GYSLNFMGYVI	XYXXXXXXXXXI	
75	15	POL	820	HFASPLHVAW	XFXXXXXXXW	
80	16	POL	7	HFRKLLLL	XFXXXXXL	
75	15	POL	435	HLLVGSSGL	XLXXXXXXL	
80	16	POL	569	HLNPNKTKRW	XLXXXXXXXW	
75	15	POL	491	HLYSHPII	XLXXXXXI	
80	16	POL	491	HLYSHPIIL	XLXXXXXXL	
80	16	POL	491	HLYSHPIILGF	XLXXXXXXXXF	
80	16	POL	715	HTAELLAACF	XTXXXXXXXF	
85	17	NUC	52	HTALRQAI	XTXXXXXI	
100	20	NUC	52	HTALRQAIL	XTXXXXXXL	
95	19	NUC	52	HTALRQAILCW	XTXXXXXXXXW	
95	19	POL	149	HTLWKAGI	XTXXXXXI	
100	20	POL	149	HTLWKAGIL	XTXXXXXXL	
100	20	POL	149	HTLWKAGILY	XTXXXXXXXY	
100	20	POL	146	HYLHTLWKAGI	XYXXXXXXXXI	
100	20	ENV	381	IFFCLWVY	XFXXXXXY	0.0087
100	20	ENV	381	IFFCLWVYI	XFXXXXXXI	
80	16	ENV	245	IFLFILLL	XFXXXXXXL	
80	16	ENV	245	IFLFILLLCL	XFXXXXXXXXL	
80	16	ENV	245	IFLFILLLCLI	XFXXXXXXXXXI	
95	19	ENV	255	IFLLVLLDY	XFXXXXXXY	
80	18	ENV	244	IIFLFILL	XIXXXXXL	
80	16	ENV	244	IIFLFILLL	XIXXXXXXL	
80	18	ENV	244	IIFLFILLLCL	XIXXXXXXXXL	
80	16	POL	497	IILGFRKI	XIXXXXXI	
80	16	POL	497	IILGFRKIPM	XIXXXXXXXM	
90	18	NUC	59	ILCWGELM	XLXXXXM	
80	16	POL	498	ILGFRKIPM	XLXXXXXXM	
100	20	ENV	249	ILLLCLIF	XLXXXXXF	
100	20	ENV	249	ILLLCLIFL	XLXXXXXXL	
100	20	ENV	249	ILLLCLIFLL	XLXXXXXXXL	
80	16	POL	760	ILRGTSFVY	XLXXXXXXY	
95	19	ENV	188	ILTIPQSL	XLXXXXXXL	
90	18	ENV	188	ILTIPQSLDSW	XLXXXXXXXXW	
90	18	POL	625	IVGLLGFAAPF	XVXXXXXXXXF	0.0004
85	17	ENV	358	IWMMWYWGPS	XWXXXXXXXXL	0.0020
95	19	POL	395	KFAVPNLQSL	XFXXXXXXXL	
80	16	POL	503	KIPMGVGL	XIXXXXXL	
80	16	POL	503	KIPMGVGLSPF	XIXXXXXXXXF	
85	17	NUC	21	KLCLGWLW	XLXXXXXXW	
85	17	NUC	21	KLCLGWLWGM	XLXXXXXXXM	
95	19	POL	489	KLHLYSHPI	XLXXXXXXI	
80	16	POL	489	KLHLYSHPII	XLXXXXXXXI	
80	18	POL	489	KLHLYSHPIIL	XLXXXXXXXXL	
75	15	POL	108	KLIMPARF	XLXXXXXF	
75	15	POL	108	KLIMPARFY	XLXXXXXY	
80	16	POL	610	KLPVNRPI	XLXXXXXI	
80	16	POL	610	KLPVNRPIDW	XLXXXXXXXW	
95	19	POL	574	KTKRWGYSL	XTXXXXXXL	
85	17	POL	574	KTKRWGYSLNF	XTXXXXXXXXF	
85	17	POL	620	KVCQRIVGL	XVXXXXXXL	
85	17	POL	620	KVCQRIVGLL	XVXXXXXXXL	
95	19	POL	55	KVGNFTGL	XVXXXXXL	

HBV A24 SUPER MOTIF (With binding information)

Conservancy	Freq	Protein	Position	Sequence	String	Peptide	A*2401
					XVXXXXXXY	1.0166	
95	19	POL	55	KVGNFTGLY	XVXXXXXL		
85	17	X	91	KVLHKRTL	XVXXXXXXXL	1.0800	0.0028
85	17	X	91	KVLHKRTLGL	XYXXXXXXI	5.0063	
100	20	POL	121	KYLPLDKGI	XYXXXXXL	17.0132	3.6000
85	17	POL	745	KYTSFPWL	XYXXXXXXL	2.0061	
85	17	POL	745	KYTSFPWLL	XFXXXXXL	17.0247	
80	16	ENV	247	LFILLLCL	XFXXXXXXI		
80	16	ENV	247	LFILLLCLI	XFXXXXXXXF		
80	16	ENV	247	LFILLLCLIF	XFXXXXXXXXL		
80	16	ENV	247	LFILLLCLIFL	XIXXXXXL	Chisari 4.014	
100	20	ENV	254	LIFLLVLL	XIXXXXXXXY	1.0899	
95	19	ENV	254	LIFLLVLLDY	XIXXXXXY	26.0028	
100	20	POL	109	LIMPARFY	XLXXXXXXI	3.0010	
95	19	POL	514	LLAQFTSAI	XLXXXXXL	Chisari 4.015	
100	20	ENV	251	LLCLIFLL	XLXXXXXXXL	1.0898	
100	20	ENV	251	LLCLIFLLVL	XLXXXXXXXXL	Chisari 4.016	
100	20	ENV	251	LLCLIFLLVLL	XLXXXXXL		
85	17	NUC	30	LLDTASAL	XLXXXXXXY	1.0155	
85	17	NUC	30	LLDTASALY	XLXXXXXL	Chisari 4.021	
95	19	ENV	260	LLDYQGML	XLXXXXXW		
80	16	POL	752	LLGCAANW	XLXXXXXXI	3.0013	
80	16	POL	752	LLGCAANWI	XLXXXXXXXL	1.0912	
80	16	POL	752	LLGCAANWIL	XLXXXXXF		
95	19	POL	628	LLGFAAPF	XLXXXXXXXXI		
75	15	ENV	63	LLGWSPQAQGI	XLXXXXXL	Chisari 4.017	
100	20	ENV	250	LLLCLIFL	XLXXXXXXL	1.0834	
100	20	ENV	250	LLLCLIFLL	XLXXXXXXXXL	Chisari 4.018	
100	20	ENV	250	LLLCLIFLLVL	XLXXXXXL	17.0112	
100	20	ENV	378	LLPIFFCL	XLXXXXXXW		
100	20	ENV	378	LLPIFFCLW	XLXXXXXXXXY	26.0549	
100	20	ENV	378	LLPIFFCLWVY	XLXXXXXXF		
95	19	NUC	44	LLSFLPSDF	XLXXXXXXXF		
95	19	NUC	44	LLSFLPSDFF	XLXXXXXL		
95	19	POL	563	LLSLGIHL	XLXXXXXW		
90	18	POL	407	LLSSNLSW	XLXXXXXXL	1.0184	
90	18	POL	407	LLSSNLSWL	XLXXXXXXXXL		
90	18	POL	407	LLSSNLSWLSL	XLXXXXXI	Chisari 4.053	
80	16	ENV	184	LLTRILTI	XLXXXXXL		
80	16	POL	436	LLVGSSGL	XLXXXXXXXM	3.0207	
95	19	ENV	257	LLVLLDYQGM	XLXXXXXXXL		
95	19	ENV	257	LLVLLDYQGML	XLXXXXXF		
95	19	ENV	175	LLVLQAGF	XLXXXXXXF	20.0121	
95	19	ENV	175	LLVLQAGFF	XLXXXXXXXL	1.0892	
90	18	ENV	175	LLVLQAGFFL	XLXXXXXXXXL	Chisari 4.028	
90	18	ENV	175	LLVLQAGFFLL	XLXXXXXW		
100	20	ENV	338	LLVPFVQW	XLXXXXXXF		
100	20	ENV	338	LLVPFVQWF	XLXXXXXXL	1.0844	
90	18	NUC	100	LLWFHISCL	XLXXXXXXXXF		
85	17	NUC	100	LLWFHISCLTF	XMXXXXXI	17.0130	
95	19	POL	643	LMPLYACI	XTXXXXXXL		
75	15	NUC	137	LTFGRETVL	XTXXXXXXXXY		
75	15	NUC	137	LTFGRETVLEY	XTXXXXXXXW		
90	18	ENV	189	LTIPQSLDSW	XTXXXXXXXXW		
90	18	ENV	189	LTIPQSLDSWW	XTXXXXXXL		
90	18	POL	404	LTNLLSSNL	XTXXXXXXXXW		
90	18	POL	404	LTNLLSSNLSW	XTXXXXXXXXL		
80	16	ENV	185	LTRILTIPQSL	XTXXXXXXL		
85	17	POL	99	LTVNEKRRL	XVXXXXXXM	3.0034	
95	19	ENV	258	LVLLDYQGM	XVXXXXXXXL	1.0515	
95	19	ENV	258	LVLLDYQGML	XVXXXXXF		
95	19	ENV	176	LVLQAGFF	XVXXXXXXL	1.0827	
90	18	ENV	176	LVLQAGFFL	XVXXXXXXXL	1.0893	
90	18	ENV	176	LVLQAGFFLL	XVXXXXXF		
100	20	ENV	339	LVPFVQWF	XVXXXXXXXXL		
95	19	ENV	339	LVPFVQWFVGL	XVXXXXXI	Chisari 4.078	
90	18	NUC	119	LVSFGVWI	XVXXXXXF		
100	20	POL	377	LVVDFSQF	XWXXXXXL		
90	18	NUC	101	LWFHISCL	XWXXXXXXXF	26.0373	
85	17	NUC	101	LWFHISCLTF	XWXXXXXXY		
85	17	NUC	27	LWGMDIDPY	XWXXXXXY		
100	20	POL	151	LWKAGILY	XYXXXXXL		1.1000
80	16	POL	492	LYSHPIIL	XYXXXXXXXF	2.0181	0.0012
80	16	POL	492	LYSHPIILGF	XMXXXXXL	1.0839	0.0001
85	17	ENV	360	MMWYWGPSL	XMXXXXXXXY	1039.01	
85	17	ENV	360	MMWYWGPSLY	XWXXXXL	17.0249	0.0027
85	17	ENV	361	MWYWGPSL	XWXXXXXY	1039.02	
85	17	ENV	361	MWYWGPSLY	XFXXXXXI		
95	19	POL	561	NFLLSLGI			

HBV A24 SUPER MOTIF (With binding information)

Conservancy	Freq	Protein	Position	Sequence	String	A*2401
						0.0099
95	19	POL	561	NFLLSLGIHL	XFXXXXXXXL	
95	19	POL	42	NLGNLNVSI	XLXXXXXXI	
95	19	POL	42	NLGNLNVSIPW	XLXXXXXXXXW	
90	18	POL	405	NLLSSNLSW	XLXXXXXXW	
90	18	POL	405	NLLSSNLSWL	XLXXXXXXXL	
95	19	POL	45	NLNVSIPW	XLXXXXXW	
100	20	POL	400	NLQSLTNL	XLXXXXXL	
100	20	POL	400	NLQSLTNLL	XLXXXXXXL	
75	15	ENV	15	NLSVPNPL	XLXXXXXXL	
75	15	ENV	15	NLSVPNPLGF	XLXXXXXXXF	
80	16	POL	758	NWILRGTSF	XWXXXXXXF	
80	16	POL	758	NWILRGTSFVY	XWXXXXXXXXY	
95	19	POL	512	PFLLAQFTSAI	XFXXXXXXXXI	0.0002
95	19	POL	534	PFTQCGYPAL	XFXXXXXXXL	
95	19	POL	534	PFTQCGYPALM	XFXXXXXXXXM	0.0003
95	19	POL	341	PPVCWFVCL	XFXXXXXXL	
95	19	ENV	615	PIDWKVCQRI	XIXXXXXXXI	
85	17	POL	380	PIFFCLWVY	XIXXXXXXY	
100	20	ENV	380	PIFFCLWVYI	XIXXXXXXXI	
100	20	POL	713	PIHTAELL	XIXXXXXL	
85	17	POL	498	PILGFRKI	XIXXXXXXI	
80	16	POL	495	PILGFRKIPM	XIXXXXXXXXM	
80	16	ENV	314	PIPSSWAF	XIXXXXXF	
100	20	POL	124	PLDKGIKPY	XLXXXXXXY	
100	20	POL	124	PLDKGIKPYY	XLXXXXXXXY	
100	20	POL	20	PLEEELPRL	XLXXXXXXL	
95	19	ENV	10	PLGFFPDHQL	XLXXXXXXXL	
95	19	POL	427	PLHPAAMPHL	XLXXXXXXXL	
100	20	POL	427	PLHPAAMPHLL	XLXXXXXXXXL	
100	20	ENV	377	PLLPIFFCL	XLXXXXXXL	
100	20	ENV	377	PLLPIFFCLW	XLXXXXXXXW	
100	20	ENV	174	PLLVLQAGF	XLXXXXXXF	
95	19	ENV	174	PLLVLQAGFF	XLXXXXXXXF	
95	19	ENV	174	PLLVLQAGFFL	XLXXXXXXXXL	
90	18	POL	711	PLPIHTAEL	XLXXXXXXL	
80	16	POL	711	PLPIHTAELL	XLXXXXXXXL	
80	16	POL	2	PLSYQHFRKL	XLXXXXXXXXL	
75	15	POL	2	PLSYQHFRKLL	XLXXXXXXXXXL	
75	15	POL	98	PLTVNEKRRL	XLXXXXXXXL	
85	17	POL	505	PMGVGLSPF	XMXXXXXXF	
80	16	POL	505	PMGVGLSPFL	XMXXXXXXXL	
80	16	POL	505	PMGVGLSPFLL	XMXXXXXXXXL	
75	15	POL	602	PTGWGLAI	XTXXXXXI	
85	17	POL	797	PTTGRTSL	XTXXXXXL	
85	17	POL	797	PTTGRTSLY	XTXXXXXXY	
80	16	NUC	15	PTVQASKL	XTXXXXXXL	
80	16	NUC	15	PTVQASKLCL	XTXXXXXXXXL	
75	15	ENV	351	PTVWLSVI	XTXXXXXI	
75	15	ENV	351	PTVWLSVIW	XTXXXXXXXW	
75	15	ENV	351	PTVWLSVIWM	XTXXXXXXXXM	
85	17	POL	612	PVNRPIDW	XVXXXXXW	
80	16	POL	750	PWLLGCAANW	XWXXXXXXXW	
80	16	POL	750	PWLLGCAANWI	XWXXXXXXXXI	0.0290
100	20	POL	51	PWTHKVGNF	XWXXXXXXF	
80	16	X	8	QLDPARDVL	XLXXXXXXL	
80	16	X	8	QLDPARDVLCL	XLXXXXXXXXL	
90	18	NUC	99	QLLWFHISCL	XLXXXXXXXXL	
95	19	POL	585	QVFADATPTGW	XVXXXXXXXXW	
95	19	ENV	344	QWPVGLSPTVW	XWXXXXXXXX	
75	15	ENV	242	RFIIFLFI	XFXXXXXI	
75	15	ENV	242	RFIIFLFIL	XFXXXXXXL	
75	15	ENV	242	RFIIFLFILL	XFXXXXXXXL	
75	15	ENV	242	RFIIFLFILLL	XFXXXXXXXXL	
100	20	ENV	332	RFSWLSLL	XFXXXXXXL	
100	20	ENV	332	RFSWLSLLVPF	XFXXXXXXXXF	
80	16	ENV	167	RILTIPQSL	XIXXXXXXL	
90	18	POL	624	RIVGLLGF	XIXXXXXXF	
75	15	POL	106	RLKLIMPARF	XLXXXXXXXF	
75	15	POL	106	RLKLIMPARFY	XLXXXXXXXXY	
95	19	POL	376	RLWWDFSQF	XLXXXXXXF	
90	18	POL	353	RTPARVTGGVF	XTXXXXXXXXF	
95	19	POL	36	RVAEDLNL	XVXXXXXL	
90	18	POL	36	RVAEDLNLGNL	XVXXXXXXXXXL	
80	16	POL	618	RVHFASPL	XVXXXXXL	
100	20	POL	357	RVTGGVFL	XVXXXXXL	
85	17	POL	577	RWGYSLNF	XWXXXXXF	
85	17	POL	577	RWGYSLNFM	XWXXXXXXM	
85	17	POL	577	RWGYSLNFMGY	XWXXXXXXXXY	

HBV A24 SUPER MOTIF (With binding Information)

Conservancy	Freq	Protein	Position	Sequence	String	A*2401
95	19	ENV	238	RWMCLRRF	XWXXXXXF	
95	19	ENV	236	RWMCLRRFI	XWXXXXXXI	0.0710
95	19	ENV	236	RWMCLRRFII	XWXXXXXXXI	1.1000
95	19	ENV	236	RWMCLRRFIIF	XWXXXXXXXXF	
100	20	POL	167	SFCGSPYSW	XFXXXXXXW	0.0710
95	19	NUC	46	SFLPSDFF	XFXXXXXF	
80	18	POL	765	SFVYVPSAL	XFXXXXXXL	
100	20	POL	49	SIPWTHKVGNF	XIXXXXXXXXF	
95	19	ENV	194	SLDSWWTSL	XLXXXXXXL	
95	19	ENV	194	SLDSWWTSLNF	XLXXXXXXXXF	
95	19	POL	416	SLDVSAAF	XLXXXXXF	
95	19	POL	416	SLDVSAAFY	XLXXXXXXY	
100	20	ENV	337	SLLVPFVQW	XLXXXXXXW	
100	20	ENV	337	SLLVPFVQWF	XLXXXXXXXF	
75	15	POL	581	SLNFMGYVI	XLXXXXXXI	
95	19	X	54	SLRGLPVCAF	XLXXXXXXXF	
90	18	POL	403	SLTNLLSSNL	XLXXXXXXXL	
75	15	X	104	STTDLEAY	XTXXXXXY	
75	15	X	104	STTDLEAYF	XTXXXXXXF	
75	15	ENV	17	SVPNPLGF	XVXXXXXF	
85	17	POL	548	SVQHLESL	XVXXXXXL	
80	16	ENV	330	SVRFSWLSL	XVXXXXXXL	
80	16	ENV	330	SVRFSWLSLL	XVXXXXXXXL	
90	18	POL	739	SVVLSRKY	XVXXXXXY	
85	17	POL	739	SVVLSRKYTSF	XVXXXXXXXXF	
95	19	POL	524	SVVRRAFPHCL	XVXXXXXXXXL	
95	19	POL	413	SWLSLDVSAAF	XWXXXXXXXXF	
100	20	ENV	334	SWLSLLVPF	XWXXXXXXF	0.3900
95	19	POL	392	SWPKFAVPNL	XWXXXXXXXL	5.6000
100	20	ENV	197	SWWTSLNF	XWXXXXXF	
95	19	ENV	197	SWWTSLNFL	XWXXXXXXL	0.3800
90	18	POL	537	SYMDDVVL	XYXXXXXL	
75	15	POL	4	SYQHFRKL	XYXXXXXL	
75	15	POL	4	SYQHFRKLL	XYXXXXXXL	0.0051
75	15	POL	4	SYQHFRKLLL	XYXXXXXXXL	0.0660
75	15	POL	4	SYQHFRKLLLL	XYXXXXXXXXL	
75	15	NUC	138	TFGRETVL	XFXXXXL	
75	15	NUC	138	TFGRETVLEY	XFXXXXXXXY	
75	15	NUC	138	TFGRETVLEYL	XFXXXXXXXXL	
95	19	POL	657	TFSPTYKAF	XFXXXXXXF	0.0080
95	19	POL	657	TFSPTYKAFL	XFXXXXXXXL	0.0043
90	18	ENV	190	TIPQSLDSW	XIXXXXXXW	
90	18	ENV	190	TIPQSLDSWW	XIXXXXXXXW	
100	20	POL	150	TLWKAGIL	XLXXXXXL	
100	20	POL	150	TLWKAGILY	XLXXXXXXY	
75	15	X	105	TTDLEAYF	XTXXXXXF	
85	17	POL	798	TTGRTSLY	XTXXXXXY	
85	17	POL	100	TVNEKRRL	XVXXXXXL	
80	16	NUC	16	TVQASKLCL	XVXXXXXXL	
80	16	NUC	16	TVQASKLCLGW	XVXXXXXXXXW	
75	15	ENV	352	TVWLSVIW	XVXXXXXW	
75	15	ENV	352	TVWLSVIWM	XVXXXXXXM	
95	19	POL	686	VFADATPTGW	XFXXXXXXXW	0.0180
75	15	X	131	VFVLGGCRHKL	XFXXXXXXXXL	
85	17	POL	543	VLGAKSVQHL	XLXXXXXXXL	
90	18	X	133	VLGGCRHKL	XLXXXXXXL	
85	17	X	92	VLHKRTLGL	XLXXXXXXL	
95	19	ENV	259	VLLDYQGM	XLXXXXXM	
95	19	ENV	259	VLLDYQGML	XLXXXXXXL	
95	19	ENV	177	VLQAGFFL	XLXXXXXL	
95	19	ENV	177	VLQAGFFLL	XLXXXXXXL	
85	17	POL	741	VLSRKYTSF	XLXXXXXXF	
85	17	POL	741	VLSRKYTSFPW	XLXXXXXXXXW	
80	16	POL	542	VVLGAKSVQHL	XVXXXXXXXXL	
85	17	POL	740	VVLSRKYTSF	XVXXXXXXXF	
95	19	POL	525	VVRRAFPHCL	XVXXXXXXXL	
95	19	NUC	124	VWIRTPPAY	XWXXXXXXY	
75	15	ENV	353	VWLSVIWM	XWXXXXXM	
90	18	NUC	102	WFHISCLTF	XFXXXXXXF	0.0300
95	19	ENV	345	WFVGLSPTVW	XFXXXXXXXW	0.0120
95	19	ENV	345	WFVGLSPTVWL	XFXXXXXXXXL	
80	16	POL	759	WILRGTSF	XIXXXXXF	
80	16	POL	759	WILRGTSFVY	XIXXXXXXXY	
95	19	NUC	125	WIRTPPAY	XIXXXXXY	
80	16	POL	751	WLLGCAANW	XLXXXXXXW	
80	16	POL	751	WLLGCAANWI	XLXXXXXXI	
80	16	POL	751	WLLGCAANWIL	XLXXXXXXXL	
95	19	POL	414	WLSLDVSAAF	XLXXXXXXXF	

HBV A24 SUPER MOTIF (With binding information)

Conservancy	Freq	Protein	Position	Sequence	String	A*2401
95	19	POL	414	WLSLDVSAAFY	XLXXXXXXXY	
100	20	ENV	335	WLSLLVPF	XLXXXXXF	
100	20	ENV	335	WLSLLVPFVQW	XLXXXXXXXW	
85	17	NUC	26	WLWGMDIDPY	XLXXXXXXY	
95	19	ENV	237	WMCLRRFI	XMXXXXXI	
95	19	ENV	237	WMCLRRFII	XMXXXXXXI	0.0230
95	19	ENV	237	WMCLRRFIIF	XMXXXXXXXF	0.0013
95	19	ENV	237	WMCLRRFIIFL	XMXXXXXXXXL	
85	17	ENV	359	WMMWYWGPSL	XMXXXXXXXL	0.0005
85	17	ENV	359	WMMWYWGPSL	XMXXXXXXXXY	
100	20	POL	52	WTHKVGNF	XTXXXXXF	
95	19	POL	52	WTHKVGNFTGL	XTXXXXXXXXL	
95	19	ENV	198	WWTSLNFL	XWXXXXXL	
85	17	ENV	362	WYWGPSLY	XYXXXXXY	0.0001
100	20	POL	147	YLHTLWKAGI	XLXXXXXXXI	
100	20	POL	147	YLHTLWKAGIL	XLXXXXXXXXL	
100	20	POL	122	YLPLDKGI	XLXXXXXI	
100	20	POL	122	YLPLDKGIKPY	XLXXXXXXXXY	
90	18	NUC	118	YLVSFGVW	XLXXXXXW	
90	18	NUC	118	YLVSFGVWI	XLXXXXXXI	
85	17	POL	746	YTSFPWLL	XTXXXXXL	

TABLE XI

HBV B07 SUPER MOTIF (With binding information)

Conservancy	Frequenc	Protein	Position	Sequence	P2	C-term	AA	B*0702	B*3501 CIR	B*5101	B*5301	B*5401
75	15	X	146	APCNFFTSA	P	A	9					
95	19	POL	633	APFTQCGY	P	Y	8	0.0001	0.0012	0.0019	0.0002	0 0002
95	19	POL	633	APFTQCGYPA	P	A	10	0.0029	0.0001		0.0002	1.4000
95	19	POL	633	APFTQCGYPAL	P	L	11	0.2300	0.0010	0.0004	-0.0003	0.0093
100	20	ENV	232	CPGYRWMCL	P	L	9					
80	16	NUC	14	CPTVQASKL	P	L	9					
80	16	NUC	14	CPTVQASKLCL	P	L	11					
80	16	X	10	DPARDVLCL	P	L	9					
80	16	ENV	122	DPRVRGLY	P	Y	8					
90	18	POL	778	DPSRGRLGL	P	L	9	0.0120	0 0001	0.0001	0.0001	0 0001
90	18	NUC	33	DPYKEFGA	P	A	8	0.0001	0.0001	0.0019	0.0002	0 0019
75	15	ENV	130	FPAGGSSSGTV	P	V	11					
90	18	ENV	14	FPDHQLDPA	P	A	9					
85	17	ENV	14	FPDHQLDPAF	P	F	10	0.0002	0.0016	0 0003	0.0011	0.0021
95	19	POL	530	FPHCLAFSY	P	Y	9	0.0001	0 5250	0.0665	0.5400	0.0199
95	19	POL	530	FPHCLAFSYM	P	M	10	0.0990	0.2200	0.0900	0.0790	0.0480
75	15	POL	749	FPWLLGCA	P	A	8					
75	15	POL	749	FPWLLGCAA	P	A	9					
75	15	POL	749	FPWLLGCAANW	P	W	11					
90	18	X	67	GPCALRFTSA	P	A	10	0.0900	0.0001	0.0001	0.0002	0.0035
95	19	POL	19	GPLEEELPRL	P	L	10	0.0001	0.0001	0.0002	0 0001	0.0002
90	18	POL	19	GPLEEELPRLA	P	A	11	-0.0002	0 0001	0.0001	-0 0003	0.0001
95	19	ENV	173	GPLLVLQA	P	A	8	0.0003	0.0001	0.0110	0.0002	0.0065
95	19	ENV	173	GPLLVLQAGF	P	F	10	0.0001	0.0001	0.0002	0.0001	0 0002
95	19	ENV	173	GPLLVLQAGFF	P	F	11	0.0011	0.0001	0.0001	0.0008	0 0009
85	17	POL	97	GPLTVNEKRRL	P	L	11	0.0031	0.0001	0.0001	-0.0003	0.0001
100	20	POL	429	HPAAMPHL	P	L	8	0.0650	0.0004	0.3100	0.0037	0.0160
100	20	POL	429	HPAAMPHLL	P	L	9	0.0980	0.0270	0.0110	0.0500	0.0120
85	17	POL	429	HPAAMPHLLV	P	V	10	0.0160	0.0020	0.0078	0.0140	0.0170
80	16	POL	495	HPIILGFRKI	P	I	10					
100	20	ENV	313	IPIPSSWA	P	A	8	0.0004	0 0004	0.0019	0.0002	0 0600
100	20	ENV	313	IPIPSSWAF	P	F	9	0.1300	2.7679	2.3500	0.7450	0.0034
80	16	ENV	313	IPIPSSWAFA	P	A	10	0.0013	0.0024		0.0014	0.4500
80	16	POL	504	IPMGVGLSPF	P	F	10					
80	16	POL	504	IPMGVGLSPFL	P	L	11					
90	18	ENV	191	IPQSLDSW	P	W	8					
90	18	ENV	191	IPQSLDSWW	P	W	9					
80	16	ENV	315	IPSSWAFA	P	A	8					
100	20	POL	50	IPWTHKVGNF	P	F	10	0.0013	0.0001	0.0007	0.0001	0 0002
100	20	ENV	379	LPIFFCLW	P	W	8	0 0001	0.0001	0.0360	0.1400	0.0035
100	20	ENV	379	LPIFFCLWV	P	V	9					
100	20	ENV	379	LPIFFCLWVY	P	Y	10	0.0002	0.0079	0.0002	0.0006	0.0002
100	20	ENV	379	LPIFFCLWVYI	P	I	11	0.0002	0.0001	0.0043	0.0139	0 0021
85	17	POL	712	LPIHTAEL	P	L	8					
85	17	POL	712	LPIHTAELL	P	L	9	0.0040	0.0630	0.0052	0 3100	0.0005
85	17	POL	712	LPIHTAELLA	P	A	10	0.0018	0.0011		0.0016	0.3300
85	17	POL	712	LPIHTAELLAA	P	A	11	0.0090	0.0027	-0.0003	0.0120	2.7500
80	16	X	89	LPKVUHKRTL	P	L	10					
100	20	POL	123	LPLDKGIKPY	P	Y	10	0.0001	0.0290	0 0002	0.0003	0.0002
100	20	POL	123	LPLDKGIKPYY	P	Y	11	-0.0002	0.0009	0.0001	0.0007	0 0001
95	19	X	58	LPVCAFSSA	P	A	9	0.0480	0.0710	0.0110	0.0009	19.0000

HBV B07 SUPER MOTIF (With binding information)

Conservancy	Frequenc	Protein	Position	Sequence	P2	C-term	AA	B*0702	B*3501 CIR	B*5101	B*5301	B*5401
80	16	POL	611	LPVNRPIDW	P	W	9					
80	16	POL	611	LPVNRPIDWKV	P	V	11					
80	16	POL	433	MPHLLVGSSGL	P	L	11					
100	20	POL	1	MPLSYQHF	P	F	8	0.0001	0.0097	0.0120	0.0370	0.0190
75	15	POL	1	MPLSYQHFRKL	P	L	11					
90	18	POL	774	NPADDPSRGRL	P	L	11	0.0120	0.0001	0.0001	-0.0003	0.0001
95	19	ENV	9	NPLGFFPDHQL	P	L	11	0.0012	0 0021	0.0001	0.0028	0.0001
75	15	POL	571	NPNKTKRW	P	W	8					
75	15	POL	571	NPNKTKRWGY	P	Y	10					
95	19	NUC	129	PPAYRPPNA	P	A	9	0.0001	0.0001	0 0001	0.0002	0.0003
95	19	NUC	129	PPAYRPPNAPI	P	I	11	0 0003	0 0001	0.0001	-0.0003	0.0001
85	17	ENV	58	PPHGGLLGW	P	W	9	0.0001	0.0002	0.0001	0.0003	0.0002
100	20	NUC	134	PPNAPILSTL	P	L	10	0.0001	0.0001	0.0035	0.0001	0.0002
80	16	POL	615	RPIDWKVCQRI	P	I	11					
100	20	NUC	133	RPPNAPIL	P	L	8	0.0076	0.0001	0.0280	0.0002	0.0002
100	20	NUC	133	RPPNAPILSTL	P	L	11	0.1300	0.0001	0.0018	-0.0003	0.0001
100	20	NUC	44	SPEHCSPHHTA	P	A	11	-0.0002	0.0001	0.0001	-0.0003	0.0011
95	19	POL	511	SPFLLAQF	P	F	8	0 5500	0.0009	0.0180	0.0009	0.0093
95	19	POL	511	SPFLLAQFTSA	P	A	11	0.0820	0.0001	0.0001	-0.0003	12.0500
100	20	NUC	49	SPHHTALRQA	P	A	10	0.0012	0.0001		0.0002	0.0035
100	20	NUC	49	SPHHTALRQAI	P	I	11	0.5800	0.0001	0.0004	0.0005	0 0002
85	17	ENV	67	SPQAQGIL	P	L	8					
85	17	POL	808	SPSVPSHL	P	L	8					
75	15	ENV	350	SPTVWLSV	P	V	8					
75	15	ENV	350	SPTVWLSVI	P	I	9					
75	15	ENV	350	SPTVWLSVIW	P	W	10					
75	15	ENV	350	SPTVWLSVIWM	P	M	11					
95	19	POL	659	SPTYKAFL	P	L	8	0.3900	0.0001	0.0019	0.0002	0.0002
90	18	POL	354	TPARVTGGV	P	V	9	0.0078	0.0001	0.0013	0.0001	0.0015
90	18	POL	354	TPARVTGGVF	P	F	10	0.3200	0 1000	0.0001	0.0099	0.0006
90	18	POL	354	TPARVTGGVFL	P	L	11	0.0950	0.0001	0.0001	0.0005	0.0005
95	19	NUC	128	TPPAYRPPNA	P	A	10	0.0001	0.0001		0.0002	0.0100
75	15	ENV	57	TPPHGGLL	P	L	8					
75	15	ENV	57	TPPHGGLLGW	P	W	10					
80	16	POL	691	TPTGWGLA	P	A	8					
75	15	POL	691	TPTGWGLAI	P	I	9					
95	19	ENV	340	VPFVQWFV	P	V	8	0.0010	0.0001	19.0000	0.0002	0.1100
95	19	ENV	340	VPFVQWFVGL	P	L	10	0.0011	0.0001	0.0100	0.0001	0.0025
95	19	POL	398	VPNLQSLTNL	P	L	10	0.0006	0.0001	0.0004	0.0001	0.0002
95	19	POL	398	VPNLQSLTNLL	P	L	11	0.0004	0.0001	0.0001	-0.0003	0.0002
90	18	POL	769	VPSALNPA	P	A	8	0.0011	0.0001	0.0070	0.0002	1.0000
95	19	POL	393	WPKFAVPNL	P	L	9	0.0054	0.0002	0.0015	0.0001	0.0015
95	19	POL	640	YPALMPLY	P	Y	8	0.0004	0.2600	0.4100	0.0450	0.0056
95	19	POL	640	YPALMPLYA	P	A	9	0 0180	0.0480	0.0340	0.0140	16.0000
95	19	POL	640	YPALMPLYACI	P	I	11	0.0040	0.0001	0 0470	0.0320	0.0700

TABLE XII

HBV B27 Super Motif

Protein	Sequence	Position in HBV	No. of Amino Acids	Sequence Frequency	Conservancy (%)
	130				
AYW	AHLSLRGL	51	8	19	95
AYW	ARVTGGVF	356	8	18	90
AYW	DHGAHLSL	48	8	19	95
AYW	DHQLDPAF	16	8	18	90
AYW	DKGIKPYY	126	8	20	100
AYW	FHISCLTF	103	8	18	90
AYW	FRKIPMGV	501	8	16	80
AYR	GRETVLEY	140	8	15	75
AYW	HHTALRQA	51	8	20	100
AYW	IHTAELLA	714	8	17	85
AYW	LHKRTLGL	93	8	18	90
AYW	LHLYSHPI	490	8	19	95
AYW	LRGLPVCA	55	8	19	95
AYW	LRGTSFVY	761	8	16	80
AYW	LRQAILCW	55	8	19	95
AYW	LRRFIIFL	240	8	19	95
AYW	NKTKRWGY	573	8	15	75
AYW	NRPIDWKV	614	8	18	90
AYW	NRRVAEDL	34	8	17	85
AYW	PHCLAFSY	531	8	19	95
AYW	PHGGLLGW	59	8	17	85
AYW	PKFAVPNL	394	8	19	95
AYR	QHFRKLLL	6	8	15	75
AYW	RHYLHTLW	145	8	20	100
AYW	RKYTSFPW	744	8	17	85
AYW	RRAFPHCL	527	8	19	95
AYW	RRFIIFLF	241	8	15	75
AYW	SHPIILGF	494	8	16	80
AYW	SKLCLGWL	20	8	18	90
AYW	SRNLYVSL	472	8	16	80
AYW	TKRWGYSL	575	8	19	95
AYW	TRHYLHTL	144	8	20	100
AYW	VRFSWLSL	331	8	16	80
AYW	WKVCQRIV	619	8	17	85
AYW	YRPPNAPI	132	8	20	100
AYW	ARVTGGVFL	356	9	18	90
AYW	EHCSPHHTA	46	9	20	100
AYR	GRETVLEYL	140	9	15	75
AYW	HHTALRQAI	51	9	20	100
AYW	HKVGNFTGL	54	9	19	95
AYW	IHTAELLAA	714	9	17	85
AYW	KRWGYSLNF	576	9	17	85
AYW	LHLYSHPII	490	9	16	80
AYW	LHPAAMPHL	428	9	20	100

HBV B27 Super Motif

Protein	Sequence	Position in HBV	No of Amino Acids	Sequence Frequency	Conservancy (%)
AYW	LHTLWKAGI	148	9	20	100
AYR	LKLIMPARF	107	9	15	75
AYW	LRGLPVCAF	55	9	19	95
AYW	LRGTSFVYV	761	9	16	80
AYW	LRRFIIFLF	240	9	15	75
AYW	PHCLAFSYM	531	9	19	95
AYW	PHHTALRQA	50	9	20	100
AYW	PKVLHKRTL	90	9	17	85
AYR	QHFRKLLLL	6	9	15	75
AYW	QRIVGLLGF	623	9	18	90
AYW	RKIPMGVGL	502	9	16	80
AYW	RKLPVNRPI	609	9	16	80
AYW	RKYTSFPWL	744	9	17	85
AYW	RRAFPHCLA	527	9	19	95
AYW	RRFIIFLFI	241	9	15	75
AYR	RRLKLIMPA	105	9	15	75
AYW	RRVAEDLNL	35	9	18	90
AYW	SKLCLGWLW	20	9	17	85
AYW	SRKYTSFPW	743	9	17	85
AYW	TRHYLHTLW	144	9	20	100
AYW	VHFASPLHV	819	9	16	80
AYW	VRFSWLSLL	331	9	16	80
AYW	VRRAFPHCL	526	9	19	95
AYW	YRPPNAPIL	132	9	20	100
AYW	YRWMCLRRF	235	9	19	95
AYW	AHLSLRGLPV	51	10	18	90
AYW	AKSVQHLESL	546	10	17	85
AYW	ARDVLCLRPV	12	10	15	75
AYW	ARVTGGVFLV	356	10	18	90
AYW	EHCSPHHTAL	46	10	20	100
AYW	FRKIPMGVGL	501	10	16	80
AYW	FRKLPVNRPI	608	10	16	80
AYR	GRETVLEYLV	140	10	15	75
AYW	HHTALRQAIL	51	10	19	95
AYW	HKVGNFTGLY	54	10	19	95
AYW	KRWGYSLNFM	576	10	17	85
AYW	LHLYSHPIIL	490	10	16	80
AYW	LHPAAMPHLL	428	10	20	100
AYW	LHTLWKAGIL	148	10	20	100
AYR	LKLIMPARFY	107	10	15	75
AYW	LRRFIIFLFI	240	10	15	75
AYW	NKTKRWGYSL	573	10	15	75
AYW	NRRVAEDLNL	34	10	17	85
AYW	PHHTALRQAI	50	10	20	100
AYW	PHLLVGSSGL	434	10	16	80

HBV B27 Super Motif

Protein	Sequence	Position in HBV	No. of Amino Acids	Sequence Frequency	Conservancy (%)
AYW	QRIVGLLGFA	623	10	18	90
AYW	RHYLHTLWKA	145	10	20	100
AYW	RKYTSFPWLL	744	10	17	85
AYW	RRAFPHCLAF	527	10	19	95
AYW	RRFIIFLFIL	241	10	15	75
AYW	SRKYTSFPWL	743	10	17	85
AYW	SRLVVDFSQF	375	10	19	95
AYW	THKVGNFTGL	53	10	19	95
AYW	TKRWGYSLNF	575	10	17	85
AYW	TKYLPLDKGI	120	10	20	100
AYW	TRILTIPQSL	186	10	16	80
AYW	VHFASPLHVA	819	10	16	80
AYW	VRFSWLSLLV	331	10	16	80
AYW	VRRAFPHCLA	526	10	19	95
AYW	WKVCQRIVGL	619	10	17	85
AYW	YRWMCLRRFI	235	10	19	95
AYW	DHGAHLSLRGL	48	11	19	95
AYW	IHLNPNKTKRW	568	11	15	75
AYW	IHTAELLAACF	714	11	17	85
AYW	LHPAAMPHLLV	428	11	17	85
AYW	LHTLWKAGILY	148	11	20	100
AYW	LRQAILCWGEL	55	11	18	90
AYW	LRRFIIFLFIL	240	11	15	75
AYW	PHHTALRQAIL	50	11	19	95
AYW	PKFAVPNLQSL	394	11	19	95
AYW	PKVLHKRTLGL	90	11	17	85
AYW	PRTPARVTGGV	352	11	18	90
AYW	QRIVGLLGFAA	623	11	18	90
AYW	RKLPVNRPIDW	609	11	16	80
AYW	RRFIIFLFILL	241	11	15	75
AYR	RRLKLIMPARF	105	11	15	75
AYW	SHPIILGFRKI	494	11	16	80
AYW	SKLCLGWLWGM	20	11	17	85
AYW	SRKYTSFPWLL	743	11	17	85
AYW	THKVGNFTGLY	53	11	19	95
AYW	TKRWGYSLNFM	575	11	17	85
AYW	TRHYLHTLWKA	144	11	20	100
AYW	VHFASPLHVAW	819	11	16	80
AYW	VRRAFPHCLAF	526	11	19	95
AYW	WKVCQRIVGLL	619	11	17	85
AYW	YRWMCLRRFII	235	11	19	95

TABLE XIII

HBV B58 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
POL	AAMPHLLV	431	8	17	85
NUC	ASALYREA	34	8	17	85
POL	ASFCGSPY	166	8	20	100
NUC	ASKLCLGW	19	8	18	90
POL	ASPLHVAW	822	8	16	80
ENV	ASVRFSWL	329	8	16	80
POL	ATPTGWGL	690	8	19	95
X	CALRFTSA	69	8	18	90
NUC	CSPHHTAL	48	8	20	100
POL	CSVVRRAF	523	8	19	95
POL	ESRLVVDF	374	8	19	95
NUC	ETVLEYLV	142	8	15	75
POL	FARSRSGA	724	8	17	85
POL	FASPLHVA	821	8	16	80
POL	FSPTYKAF	658	8	19	95
X	FSSAGPCA	63	8	19	95
ENV	FSWLSLLV	333	8	20	100
POL	FSYMDDVV	536	8	18	90
POL	FTQCGYPA	635	8	19	95
POL	FTSAICSV	518	8	19	95
POL	GAKSVQHL	545	8	17	85
POL	GTDNSVVL	735	8	18	90
POL	HTAELLAA	715	8	17	85
NUC	HTALRQAI	52	8	20	100
POL	HTLWKAGI	149	8	20	100
POL	LAQFTSAI	515	8	19	95
NUC	LSFLPSDF	45	8	19	95
POL	LSLDVSAA	415	8	19	95
ENV	LSLLVPFV	336	8	20	100
X	LSLRGLPV	53	8	19	95
POL	LSRKYTSF	742	8	17	85
POL	LSSNLSWL	408	8	18	90
POL	LSWLSLDV	412	8	20	100
NUC	LTFGRETV	108	8	19	95
X	MSTTDLEA	103	8	16	80
NUC	NAPILSTL	136	8	20	100
POL	PAAMPHLL	430	8	20	100
POL	PALMPLYA	641	8	19	95
X	PARDVLCL	11	8	16	80
POL	PARVTGGV	355	8	18	90
NUC	PAYRPPNA	130	8	19	95
POL	PSRGRLGL	779	8	18	90
POL	PTGWGLAI	692	8	15	75
POL	PTTGRTSL	797	8	17	85
NUC	PTVQASKL	15	8	16	80

HBV B58 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
ENV	PTVWLSVI	351	8	15	75
POL	RAFPHCLA	528	8	19	95
X	RTLGLSAM	96	8	24	120
NUC	SALYREAL	35	8	18	90
X	SSAGPCAL	64	8	19	95
ENV	SSGTVNPV	136	8	15	75
ENV	SSKPRQGM	5	8	18	90
NUC	STLPETTV	141	8	20	100
X	STTDLEAY	104	8	15	75
NUC	TALRQAIL	53	8	19	95
POL	TSAICSVV	519	8	19	95
ENV	TSGFLGPL	168	8	16	80
X	TTDLEAYF	105	8	15	75
POL	TTGRTSLY	798	8	17	85
POL	VSWPKFAV	391	8	19	95
NUC	VSYVNVNM	115	8	20	100
POL	VTGGVFLV	358	8	20	100
ENV	WSPQAQGI	66	8	17	85
POL	WTHKVGNF	52	8	20	100
POL	YSLNFMGY	580	8	17	85
POL	YTSFPWLL	746	8	17	85
POL	AAPFTQCGY	632	9	19	95
NUC	ASALYREAL	34	9	17	85
NUC	ASKLCLGWL	19	9	18	90
POL	ATPTGWGLA	690	9	16	80
POL	CSRNLYVSL	471	9	16	80
POL	DATPTGWGL	689	9	19	95
ENV	DSWWTSLNF	196	9	19	95
POL	EAGPLEEEL	17	9	20	100
POL	FADATPTGW	687	9	19	95
POL	FASPLHVAW	821	9	16	80
POL	FAVPNLQSL	396	9	19	95
POL	FSPTYKAFL	658	9	19	95
X	FSSAGPCAL	63	9	19	95
POL	FSYMDDVVL	536	9	18	90
POL	FTFSPTYKA	656	9	19	95
POL	FTGLYSSTV	59	9	18	90
POL	FTQCGYPAL	635	9	19	95
POL	FTSAICSVV	518	9	19	95
X	GAHLSLRGL	50	9	19	95
NUC	HTALRQAIL	52	9	19	95
POL	HTLWKAGIL	149	9	20	100
POL	KSVQHLESL	547	9	17	85
POL	KTKRWGYSL	574	9	19	95
POL	LAFSYMDDV	534	9	18	90

HBV B58 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
NUC	LSFLPSDFF	45	9	19	95
POL	LSLDVSAAF	415	9	19	95
POL	LSPFLLAQF	510	9	19	95
ENV	LSPTVWLSV	349	9	15	75
NUC	LSTLPETTV	140	9	20	100
ENV	LSVPNPLGF	16	9	15	75
POL	LSYQHFRKL	3	9	15	75
NUC	LTFGRETVL	137	9	15	75
POL	LTNLLSSNL	404	9	18	90
POL	LTVNEKRRL	99	9	17	85
X	MSTTDLEAY	103	9	15	75
POL	NSVVLSRKY	738	9	18	90
POL	PAAMPHLLV	430	9	17	85
POL	PARVTGGVF	355	9	18	90
POL	PTTGRTSLY	797	9	17	85
ENV	PTVWLSVIW	351	9	15	75
POL	QAFTFSPTY	654	9	19	95
NUC	QAILCWGEL	57	9	18	90
NUC	QASKLCLGW	18	9	16	80
POL	RAFPHCLAF	528	9	19	95
ENV	RTGDPAPNM	167	9	16	80
X	SAGPCALRF	65	9	18	90
POL	SASFCGSPY	165	9	20	100
POL	SSNLSWLSL	409	9	18	90
ENV	SSSGTVNPV	135	9	15	75
NUC	STLPETTVV	141	9	20	100
X	STTDLEAYF	104	9	15	75
POL	TAELLAACF	716	9	17	85
NUC	TASALYREA	33	9	16	80
POL	TSFVYVPSA	764	9	16	80
ENV	TSGFLGPLL	168	9	15	75
POL	TTGRTSLYA	798	9	17	85
POL	VSIPWTHKV	48	9	20	100
ENV	WSPQAQGIL	66	9	17	85
ENV	WSSKPRQGM	4	9	18	90
POL	YSHPIILGF	493	9	16	80
POL	YSLNFMGYV	580	9	15	75
POL	ASFCGSPYSW	166	10	20	100
NUC	ASKLCLGWLW	19	10	17	85
ENV	ASVRFSWLSL	329	10	16	80
POL	ATPTGWGLAI	690	10	15	75
X	CAFSSAGPCA	61	10	19	95
ENV	CTCIPIPSSW	310	10	20	100
ENV	CTIPAQGTSM	298	10	16	80
POL	DATPTGWGLA	689	10	16	80

HBV B58 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
ENV	DSWWTSLNFL	196	10	18	90
NUC	DTASALYREA	32	10	16	80
POL	FAAPFTQCGY	631	10	19	95
ENV	FSWLSLLVPF	333	10	20	100
POL	FTFSPTYKAF	656	10	19	95
POL	FTQCGYPALM	635	10	38	190
ENV	GSSSGTVNPV	134	10	15	75
ENV	GTNLSVPNPL	13	10	15	75
POL	GTSFVYVPSA	763	10	16	80
POL	HTAELLAACF	715	10	17	85
POL	HTLWKAGILY	149	10	20	100
POL	LAFSYMDDVV	534	10	18	90
POL	LSLDVSAAFY	415	10	19	95
ENV	LSLLVPFVQW	336	10	20	100
X	LSLRGLPVCA	53	10	19	95
ENV	LSPTVWLSVI	349	10	15	75
POL	LSRKYTSFPW	742	10	17	85
POL	LSSNLSWLSL	408	10	18	90
NUC	LSTLPETTVV	140	10	20	100
POL	LSWLSLDVSA	412	10	20	100
POL	LSYQHFRKLL	3	10	15	75
ENV	LTIPQSLDSW	189	10	18	90
X	MSTTDLEAYF	103	10	15	75
POL	PADDPSRGRL	775	10	18	90
ENV	PAGGSSSGTV	131	10	18	90
POL	PALMPLYACI	641	10	19	95
X	PAPCNFFTSA	145	10	15	75
POL	PARVTGGVFL	355	10	18	90
NUC	PAYRPPNAPI	130	10	19	95
POL	PTTGRTSLYA	797	10	17	85
NUC	PTVQASKLCL	15	10	16	80
ENV	PTVWLSVIWM	351	10	30	150
ENV	QAGFFLLTRI	179	10	16	80
NUC	QAILCWGELM	57	10	36	180
ENV	QAMQWNSTTF	107	10	16	80
NUC	QASKLCLGWL	18	10	16	80
ENV	QSLDSWWTSL	193	10	18	90
POL	RTPARVTGGV	353	10	18	90
POL	SAICSVVRRA	520	10	19	95
X	SSAGPCALRF	64	10	18	90
POL	TAELLAACFA	716	10	17	85
NUC	TALRQAILCW	53	10	19	95
NUC	TASALYREAL	33	10	16	80
POL	TSFPWLLGCA	747	10	15	75
POL	TSFVYVPSAL	764	10	16	80

HBV B58 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
ENV	TSGFLGPLLV	168	10	15	75
POL	VAEDLNLGNL	37	10	19	95
POL	YSLNFMGYVI	580	10	15	75
POL	AACFARSRSGA	721	11	17	85
POL	AAPFTQCGYPA	632	11	19	95
ENV	ASVRFSWLSLL	329	11	16	80
X	CAFSSAGPCAL	61	11	19	95
X	CALRFTSARRM	69	11	26	130
NUC	CSPHHTALRQA	48	11	20	100
ENV	CTCIPIPSSWA	310	11	20	100
POL	DATPTGWGLAI	689	11	15	75
NUC	DTASALYREAL	32	11	16	80
POL	ESRLVVDFSQF	374	11	19	95
POL	FADATPTGWGL	687	11	19	95
X	FSSAGPCALRF	63	11	18	90
ENV	FSWLSLLVPFV	333	11	20	100
POL	FSYMDDVVLGA	536	11	18	90
POL	FTFSPTYKAFL	656	11	19	95
X	GAHLSLRGLPV	50	11	18	90
POL	GAKSVQHLESL	545	11	17	85
POL	GTSFVYVPSAL	763	11	16	80
POL	HTAELLAACFA	715	11	17	85
NUC	HTALRQAILCW	52	11	19	95
NUC	ISCLTFGRETV	105	11	18	90
POL	KTKRWGYSLNF	574	11	17	85
POL	LAFSYMDDVVL	534	11	18	90
POL	LAQFTSAICSV	515	11	19	95
ENV	LSLLVPFVQWF	336	11	20	100
X	LSLRGLPVCAF	53	11	19	95
ENV	LSPTVWLSVIW	349	11	15	75
POL	LSRKYTSFPWL	742	11	17	85
POL	LSWLSLDVSAA	412	11	19	95
POL	LSYQHFRKLLL	3	11	15	75
NUC	LTFGRETVLEY	137	11	15	75
ENV	LTIPQSLDSWW	189	11	18	90
POL	LTNLLSSNLSW	404	11	18	90
ENV	LTRILTIPQSL	185	11	16	80
X	PARDVLCLRPV	11	11	15	75
POL	PARVTGGVFLV	355	11	18	90
NUC	PAYRPPNAPIL	130	11	19	95
ENV	PTVWLSVIWMM	351	11	28	140
POL	QAFTFSPTYKA	654	11	19	95
ENV	QAGFFLLTRIL	179	11	16	80
NUC	QASKLCLGWLW	18	11	15	75
POL	QSLTNLLSSNL	402	11	18	90

HBV B58 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
POL	RAFPHCLAFSY	528	11	19	95
POL	RTPARVTGGVF	353	11	18	90
NUC	RTPPAYRPPNA	127	11	19	95
POL	SAICSVVRRAF	520	11	19	95
POL	SASFCGSPYSW	165	11	20	100
POL	SSNLSWLSLDV	409	11	18	90
POL	TSAICSVVRRA	519	11	19	95
POL	TSFPWLLGCAA	747	11	15	75
ENV	TSGFLGPLLVL	168	11	15	75
POL	VSWPKFAVPNL	391	11	19	95
POL	WTHKVGNFTGL	52	11	19	95
POL	YTSFPWLLGCA	746	11	15	75
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TABLE XIV

HBV B62 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
NUC	AILCWGEL	58	8	18	90
POL	APFTQCGY	633	8	19	95
POL	AVPNLQSL	397	8	19	95
ENV	CIPIPSSW	312	8	20	100
NUC	CLGWLWGM	23	8	17	85
ENV	CLIFLLVL	253	8	20	100
ENV	CLRRFIIF	239	8	19	95
POL	CQRIVGLL	622	8	17	85
NUC	DIDPYKEF	31	8	18	90
NUC	DLLDTASA	29	8	17	85
ENV	DPRVRGLY	122	8	16	80
NUC	DPYKEFGA	33	8	18	90
X	DVLCLRPV	14	8	19	95
X	ELGEEIRL	122	8	16	80
POL	ELLAACFA	718	8	18	90
ENV	FIIFLFIL	243	8	16	80
ENV	FILLLCLI	248	8	16	80
ENV	FLGPLLVL	171	8	15	75
ENV	FLLVLLDY	256	8	19	95
POL	FPWLLGCA	749	8	15	75
ENV	FVGLSPTV	346	8	19	95
ENV	FVQWFVGL	342	8	19	95
POL	FVYVPSAL	766	8	18	90
POL	GLSPFLLA	509	8	19	95
ENV	GLSPTVWL	348	8	20	100
ENV	GMLPVCPL	265	8	18	90
ENV	GPLLVLQA	173	8	19	95
POL	GVGLSPFL	507	8	16	80
POL	HLYSHPII	491	8	16	80
POL	HPAAMPHL	429	8	20	100
ENV	IIFLFILL	244	8	16	80
POL	IILGFRKI	497	8	16	80
NUC	ILCWGELM	59	8	18	90
ENV	ILLLCLIF	249	8	20	100
POL	ILRGTSFV	760	8	16	80
ENV	ILTIPQSL	188	8	19	95
ENV	IPIPSSWA	313	8	20	100
ENV	IPQSLDSW	191	8	18	90
ENV	IPSSWAFA	315	8	16	80
POL	IVGLLGFA	625	8	18	90
POL	KIPMGVGL	503	8	16	80
NUC	KLCLGWLW	21	8	17	85
POL	KLIMPARF	108	8	15	75
POL	KLPVNRPI	610	8	16	80
POL	KVGNFTGL	55	8	19	95

HBV B62 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
X	KVLHKRTL	91	8	17	85
ENV	LIFLLVLL	254	8	20	100
POL	LIMPARFY	109	8	20	100
POL	LLAQFTSA	514	8	19	95
ENV	LLCLIFLL	251	8	20	100
NUC	LLDTASAL	30	8	17	85
ENV	LLDYQGML	260	8	19	95
POL	LLGCAANW	752	8	16	80
POL	LLGFAAPF	628	8	19	95
ENV	LLGWSPQA	63	8	17	85
ENV	LLLCLIFL	250	8	20	100
ENV	LLPIFFCL	378	8	20	100
POL	LLSLGIHL	563	8	19	95
POL	LLSSNLSW	407	8	18	90
ENV	LLTRILTI	184	8	16	80
POL	LLVGSSGL	436	8	16	80
ENV	LLVLQAGF	175	8	19	95
ENV	LLVPFVQW	338	8	20	100
POL	LMPLYACI	643	8	19	95
ENV	LPIFFCLW	379	8	20	100
POL	LPIHTAEL	712	8	17	85
ENV	LQAGFFLL	178	8	19	95
POL	LQSLTNLL	401	8	20	100
ENV	LVLQAGFF	176	8	19	95
ENV	LVPFVQWF	339	8	20	100
NUC	LVSFGVWI	119	8	18	90
POL	LVVDFSQF	377	8	20	100
POL	MPLSYQHF	1	8	20	100
NUC	MQLFHLCL	1	8	15	75
ENV	MQWNSTTF	109	8	16	80
POL	NLNVSIPW	45	8	19	95
POL	NLQSLTNL	400	8	20	100
ENV	NLSVPNPL	15	8	15	75
POL	NPNKTKRW	571	8	15	75
ENV	PIFFCLWV	380	8	20	100
POL	PIHTAELL	713	8	17	85
ENV	PIPSSWAF	314	8	20	100
ENV	PQSLDSWW	192	8	18	90
X	PVCAFSSA	59	8	19	95
POL	PVNRPIDW	612	8	17	85
X	QLDPARDV	8	8	16	80
POL	RIVGLLGF	624	8	18	90
POL	RLKLIMPA	106	8	15	75
NUC	RPPNAPIL	133	8	20	100
NUC	RQLLWFHI	98	8	18	90

HBV B62 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
POL	RVAEDLNL	36	8	19	95
POL	RVHFASPL	818	8	16	80
POL	RVTGGVFL	357	8	20	100
POL	SIPWTHKV	49	8	20	100
POL	SLDVSAAF	416	8	19	95
POL	SLNFMGYV	581	8	15	75
POL	SPFLLAQF	511	8	19	95
ENV	SPQAQGIL	67	8	17	85
POL	SPSVPSHL	808	8	17	85
ENV	SPTVWLSV	350	8	15	75
POL	SPTYKAFL	659	8	19	95
ENV	SVPNPLGF	17	8	15	75
POL	SVQHLESL	548	8	17	85
POL	SVVLSRKY	739	8	18	90
NUC	TLPETTVV	142	8	20	100
POL	TLWKAGIL	150	8	20	100
ENV	TPPHGGLL	57	8	15	75
POL	TPTGWGLA	691	8	16	80
POL	TQCGYPAL	636	8	19	95
POL	TVNEKRRL	100	8	17	85
ENV	TVWLSVIW	352	8	15	75
ENV	VLLDYQGM	259	8	19	95
ENV	VLQAGFFL	177	8	19	95
ENV	VPFVQWFV	340	8	19	95
POL	VPSALNPA	769	8	18	90
NUC	VQASKLCL	17	8	16	80
POL	VVLGAKSV	542	8	18	90
POL	WILRGTSF	759	8	16	80
NUC	WIRTPPAY	125	8	19	95
POL	WLSLDVSA	414	8	20	100
ENV	WLSLLVPF	335	8	20	100
ENV	WMCLRRFI	237	8	19	95
POL	YLHTLWKA	147	8	20	100
POL	YLPLDKGI	122	8	20	100
NUC	YLVSFGVW	118	8	18	90
POL	YPALMPLY	640	8	19	95
POL	YQHFRKLL	5	8	15	75
POL	AICSVVRRA	521	9	19	95
NUC	AILCWGELM	58	9	18	90
POL	ALMPLYACI	642	9	19	95
NUC	ALRQAILCW	54	9	19	95
ENV	AMQWNSTTF	108	9	16	80
X	AMSTTDLEA	102	9	15	75
X	APCNFFTSA	146	9	15	75
ENV	CIPIPSSWA	312	9	20	100

HBV B62 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
ENV	CLIFLLVLL	253	9	20	100
ENV	CLRRFIIFL	239	9	19	95
NUC	CLTFGRETV	107	9	18	90
ENV	CPGYRWMCL	232	9	20	100
NUC	CPTVQASKL	14	9	16	80
X	CQLDPARDV	7	9	16	80
NUC	DLLDTASAL	29	9	17	85
POL	DLNLGNLNV	40	9	19	95
X	DPARDVLCL	10	9	16	80
POL	DPSRGRLGL	778	9	18	90
POL	DVVLGAKSV	541	9	18	90
ENV	FIIFLFILL	243	9	16	80
ENV	FILLLCLIF	248	9	16	80
ENV	FLFILLLCL	246	9	16	80
POL	FLLAQFTSA	513	9	19	95
POL	FLLSLGIHL	562	9	19	95
ENV	FLLTRILTI	183	9	16	80
ENV	FPDHQLDPA	14	9	18	90
POL	FPHCLAFSY	530	9	19	95
POL	FPWLLGCAA	749	9	15	75
ENV	FVGLSPTVW	346	9	19	95
POL	GLCQVFADA	682	9	17	85
POL	GLLGFAAPF	627	9	19	95
ENV	GLLGWSPQA	62	9	17	85
POL	GVGLSPFLL	507	9	16	80
NUC	GVWIRTPPA	123	9	19	95
POL	HLLVGSSGL	435	9	16	80
X	HLSLRGLPV	52	9	18	90
POL	HLYSHPIIL	491	9	16	80
POL	HPAAMPHLL	429	9	20	100
ENV	IIFLFILLL	244	9	16	80
POL	ILGFRKIPM	498	9	16	80
ENV	ILLLCLIFL	249	9	20	100
POL	ILRGTSFVY	760	9	16	80
ENV	IPIPSSWAF	313	9	20	100
ENV	IPQSLDSWW	191	9	18	90
POL	IVGLLGFAA	625	9	18	90
POL	KLHLYSHPI	489	9	19	95
POL	KLIMPARFY	108	9	15	75
POL	KVCQRIVGL	620	9	17	85
POL	KVGNFTGLY	55	9	19	95
POL	LLAQFTSAI	514	9	19	95
ENV	LLCLIFLLV	251	9	20	100
NUC	LLDTASALY	30	9	17	85
POL	LLGCAANWI	752	9	16	80

HBV B62 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
ENV	LLLCLIFLL	250	9	20	100
ENV	LLPIFFCLW	378	9	20	100
NUC	LLSFLPSDF	44	9	19	95
POL	LLSSNLSWL	407	9	18	90
ENV	LLVLQAGFF	175	9	19	95
ENV	LLVPFVQWF	338	9	20	100
NUC	LLWFHISCL	100	9	18	90
ENV	LPIFFCLWV	379	9	20	100
POL	LPIHTAELL	712	9	17	85
X	LPVCAFSSA	58	9	19	95
POL	LPVNRPIDW	611	9	16	80
ENV	LVLLDYQGM	258	9	19	95
ENV	LVLQAGFFL	176	9	18	90
ENV	LVPFVQWFV	339	9	19	95
ENV	MMWYWGPSL	360	9	17	85
POL	NLGNLNVSI	42	9	19	95
POL	NLLSSNLSW	406	9	18	90
POL	NLQSLTNLL	400	9	20	100
POL	NLSWLSLDV	411	9	18	90
ENV	PIFFCLWVY	380	9	20	100
POL	PIHTAELLA	713	9	17	85
POL	PILGFRKI	496	9	16	80
ENV	PIPSSWAFA	314	9	16	80
POL	PLDKGIKPY	124	9	20	100
POL	PLEEELPRL	20	9	19	95
ENV	PLLPIFFCL	377	9	20	100
ENV	PLLVLQAGF	174	9	19	95
POL	PLPIHTAEL	711	9	16	80
POL	PMGVGLSPF	505	9	16	80
NUC	PPAYRPPNA	129	9	19	95
ENV	PPHGGLLGW	58	9	17	85
X	QLDPARDVL	8	9	16	80
ENV	RILTIPQSL	187	9	16	80
POL	RIVGLLGFA	624	9	18	90
POL	RLVVDFSQF	376	9	19	95
POL	RVTGGVFLV	357	9	20	100
ENV	SLDSWWTSL	194	9	19	95
POL	SLDVSAAFY	416	9	19	95
ENV	SLLVPFVQW	337	9	20	100
POL	SLNFMGYVI	581	9	15	75
X	SLRGLPVCA	54	9	19	95
ENV	SPTVWLSVI	350	9	15	75
ENV	SVRFSWLSL	330	9	16	80
ENV	TIPQSLDSW	190	9	18	90
POL	TLWKAGILY	150	9	20	100

HBV B62 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
POL	TPARVTGGV	354	9	18	90
POL	TPTGWGLAI	691	9	15	75
POL	TQCGYPALM	636	9	19	95
NUC	TVQASKLCL	16	9	16	80
ENV	TVWLSVIWM	352	9	15	75
X	VLCLRPVGA	15	9	19	95
X	VLGGCRHKL	133	9	18	90
X	VLHKRTLGL	92	9	17	85
ENV	VLLDYQGML	259	9	19	95
ENV	VLQAGFFLL	177	9	19	95
POL	VLSRKYTSF	741	9	17	85
POL	WILRGTSFV	759	9	16	80
POL	WLLGCAANW	751	9	16	80
POL	WLSLDVSAA	414	9	19	95
ENV	WLSLLVPFV	335	9	20	100
ENV	WMCLRRFII	237	9	19	95
POL	WPKFAVPNL	393	9	19	95
NUC	YLVSFGVWI	118	9	18	90
POL	YMDDVVLGA	538	9	18	90
POL	YPALMPLYA	640	9	19	95
POL	YQHFRKLLL	5	9	15	75
POL	YVPSALNPA	768	9	18	90
POL	AICSVVRRAF	521	10	19	95
POL	APFTQCGYPA	633	10	19	95
POL	AQFTSAICSV	516	10	19	95
ENV	CIPIPSSWAF	312	10	20	100
POL	CLAFSYMDDV	533	10	18	90
NUC	CLGWLWGMDI	23	10	17	85
ENV	CLRRFIIFLF	239	10	15	75
X	CQLDPARDVL	7	10	16	80
POL	CQRIVGLLGF	622	10	17	85
NUC	DIDPYKEFGA	31	10	18	90
NUC	DLLDTASALY	29	10	17	85
X	DVLCLRPVGA	14	10	19	95
NUC	ELLSFLPSDF	43	10	19	95
ENV	FIIFLFILLL	243	10	16	80
ENV	FILLLCLIFL	248	10	16	80
ENV	FLFILLLCLI	246	10	16	80
ENV	FLGPLLVLQA	171	10	15	75
POL	FLLAQFTSAI	513	10	19	95
ENV	FPDHQLDPAF	14	10	17	85
POL	FPHCLAFSYM	530	10	19	95
ENV	FVGLSPTVWL	346	10	19	95
X	FVLGGCRHKL	132	10	18	90
X	GLPVCAFSSA	57	10	19	95

HBV B62 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
POL	GLSPFLLAQF	509	10	19	95
ENV	GLSPTVWLSV	348	10	15	75
NUC	GMDIDPYKEF	29	10	17	85
X	GPCALRFTSA	67	10	18	90
POL	GPLEEELPRL	19	10	19	95
ENV	GPLLVLQAGF	173	10	19	95
POL	GVGLSPFLLA	507	10	16	80
NUC	GVWIRTPPAY	123	10	19	95
POL	HLNPNKTKRW	569	10	15	75
POL	HPAAMPHLLV	429	10	17	85
POL	HPIILGFRKI	495	10	16	80
POL	IILGFRKIPM	497	10	16	80
ENV	ILLLCLIFLL	249	10	20	100
POL	ILRGTSFVYV	760	10	16	80
NUC	ILSTLPETTV	139	10	20	100
ENV	IPIPSSWAFA	313	10	16	80
POL	IPMGVGLSPF	504	10	16	80
POL	IPWTHKVGNF	50	10	20	100
NUC	KLCLGWLWGM	21	10	17	85
POL	KLHLYSHPII	489	10	16	80
POL	KLPVNRPIDW	610	10	16	80
POL	KQAFTFSPTY	653	10	19	95
POL	KVCQRIVGLL	620	10	17	85
X	KVLHKRTLGL	91	10	17	85
ENV	LIFLLVLLDY	254	10	19	95
ENV	LLCLIFLLVL	251	10	20	100
ENV	LLDYQGMLPV	260	10	18	90
POL	LLGCAANWIL	752	10	16	80
ENV	LLLCLIFLLV	250	10	20	100
ENV	LLPIFFCLWV	378	10	20	100
NUC	LLSFLPSDFF	44	10	19	95
ENV	LLVLLDYQGM	257	10	19	95
ENV	LLVLQAGFFL	175	10	18	90
ENV	LLVPFVQWFV	338	10	19	95
ENV	LPIFFCLWVY	379	10	20	100
POL	LPIHTAELLA	712	10	17	85
X	LPKVLHKRTL	89	10	16	80
POL	LPLDKGIKPY	123	10	20	100
ENV	LVLLDYQGML	258	10	19	95
ENV	LVLQAGFFLL	176	10	18	90
ENV	MMWYWGPSLY	360	10	17	85
POL	NLLSSNLSWL	406	10	18	90
ENV	NLSVPNPLGF	15	10	15	75
POL	NPNKTKRWGY	571	10	15	75
POL	NVSIPWTHKV	47	10	20	100

HBV B62 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
POL	PIDWKVCQRI	616	10	17	85
ENV	PIFFCLWVYI	380	10	20	100
POL	PIHTAELLAA	713	10	17	85
POL	PLDKGIKPYY	124	10	20	100
POL	PLEEELPRLA	20	10	18	90
ENV	PLGFFPDHQL	10	10	19	95
POL	PLHPAAMPHL	427	10	20	100
ENV	PLLPIFFCLW	377	10	20	100
ENV	PLLVLQAGFF	174	10	19	95
POL	PLPIHTAELL	711	10	16	80
POL	PLSYQHFRKL	2	10	15	75
POL	PLTVNEKRRL	98	10	17	85
POL	PMGVGLSPFL	505	10	16	80
NUC	PPNAPILSTL	134	10	20	100
POL	PVNRPIDWKV	612	10	17	85
NUC	QLLWFHISCL	99	10	18	90
POL	RIVGLLGFAA	624	10	18	90
POL	RLKLIMPARF	106	10	15	75
NUC	RQAILCWGEL	56	10	18	90
POL	RVHFASPLHV	818	10	15	75
ENV	SLLVPFVQWF	337	10	20	100
X	SLRGLPVCAF	54	10	19	95
POL	SLTNLLSSNL	403	10	18	90
NUC	SPHHTALRQA	49	10	20	100
ENV	SPTVWLSVIW	350	10	15	75
ENV	SVRFSWLSLL	330	10	16	80
ENV	TIPQSLDSWW	190	10	18	90
POL	TPARVTGGVF	354	10	18	90
NUC	TPPAYRPPNA	128	10	19	95
ENV	TPPHGGLLGW	57	10	15	75
POL	VLGAKSVQHL	543	10	17	85
X	VLGGCRHKLV	133	10	18	90
ENV	VPFVQWFVGL	340	10	19	95
POL	VPNLQSLTNL	398	10	19	95
NUC	VQASKLCLGW	17	10	16	80
POL	VVLSRKYTSF	740	10	17	85
POL	VVRRAFPHCL	525	10	19	95
POL	WILRGTSFVY	759	10	16	80
POL	WLLGCAANWI	751	10	16	80
POL	WLSLDVSAAF	414	10	19	95
NUC	WLWGMDIDPY	26	10	17	85
ENV	WMCLRRFIIF	237	10	19	95
ENV	WMMWYWGPSL	359	10	17	85
POL	YLHTLWKAGI	147	10	20	100
ENV	YQGMLPVCPL	263	10	18	90

HBV B62 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
POL	YQHFRKLLLL	5	10	15	75
POL	APFTQCGYPAL	633	11	19	95
POL	AQFTSAICSVV	516	11	19	95
POL	AVPNLQSLTNL	397	11	19	95
ENV	CIPIPSSWAFA	312	11	16	80
POL	CLAFSYMDDVV	533	11	18	90
ENV	CLIFLLVLLDY	253	11	19	95
ENV	CLRRFIIFLFI	239	11	15	75
NUC	CPTVQASKLCL	14	11	16	80
POL	CQRIVGLLGFA	622	11	17	85
POL	DLNLGNLNVSI	40	11	19	95
NUC	ELLSFLPSDFF	43	11	19	95
ENV	FILLLCLIFLL	248	11	16	80
ENV	FLFILLLCLIF	246	11	16	80
ENV	FLLVLLDYQGM	256	11	19	95
ENV	FPAGGSSSGTV	130	11	15	75
POL	FPWLLGCAANW	749	11	15	75
X	FVLGGCRHKLV	132	11	18	90
POL	FVYVPSALNPA	766	11	18	90
ENV	GLSPTVWLSVI	348	11	15	75
POL	GPLEEELPRLA	19	11	18	90
ENV	GPLLVLQAGFF	173	11	19	95
POL	GPLTVNEKRRL	97	11	17	85
X	HLSLRGLPVCA	52	11	18	90
POL	HLYSHPIILGF	491	11	16	80
ENV	IIFLFILLLCL	244	11	16	80
ENV	ILLLCLIFLLV	249	11	20	100
NUC	ILSTLPETTVV	139	11	20	100
ENV	ILTIPQSLDSW	188	11	18	90
POL	IPMGVGLSPFL	504	11	16	80
POL	IVGLLGFAAPF	625	11	18	90
POL	KIPMGVGLSPF	503	11	16	80
POL	KLHLYSHPIIL	489	11	16	80
ENV	LLCLIFLLVLL	251	11	20	100
ENV	LLGWSPQAQGI	63	11	15	75
ENV	LLLCLIFLLVL	250	11	20	100
ENV	LLPIFFCLWVY	378	11	20	100
POL	LLSSNLSWLSL	407	11	18	90
ENV	LLVLLDYQGML	257	11	19	95
ENV	LLVLQAGFFLL	175	11	18	90
NUC	LLWFHISCLTF	100	11	17	85
ENV	LPIFFCLWVYI	379	11	20	100
POL	LPIHTAELLAA	712	11	17	85
POL	LPLDKGIKPYY	123	11	20	100
POL	LPVNRPIDWKV	611	11	16	80

HBV B62 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
ENV	LQAGFFLLTRI	178	11	16	80
ENV	LVPFVQWFVGL	339	11	19	95
POL	MPHLLVGSSGL	433	11	16	80
POL	MPLSYQHFRKL	1	11	15	75
POL	NLGNLNVSIPW	42	11	19	95
POL	NLSWLSLDVSA	411	11	18	90
POL	NPADDPSRGRL	774	11	18	90
ENV	NPLGFFPDHQL	9	11	19	95
POL	PIDWKVCQRIV	616	11	17	85
POL	PIILGFRKIPM	496	11	16	80
NUC	PILSTLPETTV	138	11	20	100
POL	PLHPAAMPHLL	427	11	20	100
ENV	PLLPIFFCLWV	377	11	20	100
ENV	PLLVLQAGFFL	174	11	18	90
POL	PLPIHTAELLA	711	11	16	80
POL	PLSYQHFRKLL	2	11	15	75
POL	PMGVGLSPFLL	505	11	16	80
NUC	PPAYRPPNAPI	129	11	19	95
ENV	PQAMQWNSTTF	106	11	16	80
ENV	PQSLDSWWTSL	192	11	18	90
X	QLDPARDVLCL	8	11	16	80
POL	QVFADATPTGW	685	11	19	95
POL	RLKLIMPARFY	106	11	15	75
POL	RPIDWKVCQRI	615	11	16	80
NUC	RPPNAPILSTL	133	11	20	100
NUC	RQAILCWGELM	56	11	18	90
NUC	RQLLWFHISCL	98	11	18	90
POL	RVAEDLNLGNL	36	11	18	90
POL	RVHFASPLHVA	818	11	15	75
POL	SIPWTHKVGNF	49	11	20	100
ENV	SLDSWWTSLNF	194	11	19	95
ENV	SLLVPFVQWFV	337	11	19	95
NUC	SPEHCSPHHTA	44	11	20	100
POL	SPFLLAQFTSA	511	11	19	95
NUC	SPHHTALRQAI	49	11	20	100
ENV	SPTVWLSVIWM	350	11	15	75
ENV	SVRFSWLSLLV	330	11	16	80
POL	SVVLSRKYTSF	739	11	17	85
POL	SVVRRAFPHCL	524	11	19	95
POL	TPARVTGGVFL	354	11	18	90
POL	TQCGYPALMPL	636	11	19	95
NUC	TVQASKLCLGW	16	11	16	80
ENV	VLLDYQGMLPV	259	11	18	90
POL	VLSRKYTSFPW	741	11	17	85
POL	VPNLQSLTNLL	398	11	19	95

HBV B62 Super Motif

Protein	Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
NUC	VQASKLCLGWL	17	11	16	80
ENV	VQWFVGLSPTV	343	11	19	95
POL	VVLGAKSVQHL	542	11	16	80
POL	VVRRAFPHCLA	525	11	19	95
POL	WILRGTSFVYV	759	11	16	80
POL	WLLGCAANWIL	751	11	16	80
POL	WLSLDVSAAFY	414	11	19	95
ENV	WLSLLVPFVQW	335	11	20	100
ENV	WMCLRRFIIFL	237	11	19	95
ENV	WMMWYWGPSLY	359	11	17	85
POL	YLHTLWKAGIL	147	11	20	100
POL	YLPLDKGIKPY	122	11	20	100
POL	YPALMPLYACI	640	11	19	95

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Table XV

HBV A01 Motif with Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0101
100	20	POL	166	ASFCGSPY	8	
90	18	POL	737	DNSVVLSRKY	10	0.0001
95	19	POL	631	FAAPFTQCGY	10	0.0680
95	19	POL	630	GFAAPFTQCGY	11	
75	15	NUC	140	GRETVLEY	8	
85	17	POL	579	GYSLNFMGY	9	
100	20	POL	149	HTLWKAGILY	10	0.1100
95	19	POL	653	KQAFTFSPTY	10	0.0001
85	17	NUC	30	LLDTASALY	9	12.0000
95	19	POL	415	LSLDVSAAFY	10	0.0150
75	15	NUC	137	LTFGRETVLEY	11	
85	17	ENV	360	MMWYWGPSLY	10	0.0810
75	15	X	103	MSTTDLEAY	9	0.8500
90	18	POL	738	NSVVLSRKY	9	0.0005
100	20	POL	124	PLDKGIKPY	9	
100	20	POL	124	PLDKGIKPYY	10	0.1700
85	17	POL	797	PTTGRTSLY	9	0.2100
100	20	POL	165	SASFCGSPY	9	
95	19	POL	416	SLDVSAAFY	9	5.2000
75	15	X	104	STTDLEAY	8	
85	17	POL	798	TTGRTSLY	8	
95	19	POL	414	WLSLDVSAAFY	11	
85	17	ENV	359	WMMWYWGPSLY	11	0.3200
95	19	POL	640	YPALMPLY	8	
85	17	POL	580	YSLNFMGY	8	

TABLE XVI

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
85	17	POL	721	AACFARSR	8	0.0004
85	17	POL	721	AACFARSRSGA	11	
95	19	POL	632	AAPFTQCGY	9	
95	19	POL	632	AAPFTQCGYPA	11	
85	17	POL	722	ACFARSRSGA	10	
80	16	POL	688	ADATPTGWGLA	11	
90	18	POL	776	ADDPSRGR	8	
95	19	POL	529	AFPHCLAF	8	
95	19	POL	529	AFPHCLAFSY	10	
95	19	X	62	AFSSAGPCA	9	
90	18	X	62	AFSSAGPCALR	11	
95	19	POL	655	AFTFSPTY	8	
95	19	POL	655	AFTFSPTYK	9	0.2600
95	19	POL	655	AFTFSPTYKA	10	
95	19	POL	655	AFTFSPTYKAF	11	
80	16	ENV	180	AGFFLLTR	8	
90	18	X	66	AGPCALRF	8	
90	18	X	66	AGPCALRFTSA	11	
95	19	POL	18	AGPLEEELPR	10	0.0004
95	19	POL	521	AICSVVRR	8	-0.0002
95	19	POL	521	AICSVVRRA	9	
95	19	POL	521	AICSVVRRAF	10	
95	19	NUC	41	ALESPEHCSPH	11	
90	18	POL	772	ALNPADDPSR	10	0.0003
85	17	X	70	ALRFTSAR	8	0.0047
80	16	ENV	108	AMQWNSTTF	9	
80	16	ENV	108	AMQWNSTTFH	10	
75	15	X	102	AMSTTDLEA	9	
85	17	NUC	34	ASALYREA	8	
100	20	POL	166	ASFCGSPY	8	0.0460
80	16	POL	822	ASPLHVAWR	9	
75	15	ENV	84	ASTNRQSGR	9	0.0009
80	16	POL	690	ATPTGWGLA	9	
80	16	POL	755	CAANWILR	8	
95	19	X	61	CAFSSAGPCA	10	
90	18	X	69	CALRFTSA	8	
85	17	X	69	CALRFTSAR	9	0.0034
80	16	X	6	CCOLDPAR	8	
85	17	POL	723	CFARSRSGA	9	
75	15	POL	607	CFRKLPVNR	9	
95	19	POL	638	CGYPALMPLY	10	
95	19	POL	638	CGYPALMPLYA	11	

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
100	20	ENV	312	CIPIPSSWA	9	
100	20	ENV	312	CIPIPSSWAF	10	
80	16	ENV	312	CIPIPSSWAFA	11	
95	19	ENV	253	CLIFLLVLLDY	11	0.0083
90	18	X	17	CLRPVGAESR	10	0.0011
95	19	ENV	239	CLRRFIIF	8	
75	15	ENV	239	CLRRFIIFLF	10	
100	20	NUC	48	CSPHHTALR	9	0.0029
100	20	NUC	48	CSPHHTALRQA	11	
95	19	POL	523	CSVVRRAF	8	
95	19	POL	523	CSVVRRAFPH	10	
100	20	ENV	310	CTCIPIPSSWA	11	
80	16	POL	689	DATPTGWGLA	10	
90	18	POL	540	DDVVLGAK	8	
90	18	NUC	31	DIDPYKEF	8	
90	18	NUC	31	DIDPYKEFGA	10	
85	17	NUC	29	DLLDTASA	8	
85	17	NUC	29	DLLDTASALY	10	0.0001
85	17	NUC	29	DLLDTASALYR	11	0.0042
95	19	ENV	196	DSWWTSLNF	9	0.0006
85	17	NUC	32	DTASALYR	8	0.0004
80	16	NUC	32	DTASALYREA	10	
95	19	X	14	DVLCLRPVGA	10	
95	19	POL	418	DVSAAFYH	8	
90	18	POL	541	DVVLGAKSVQH	11	
95	19	POL	17	EAGPLEEELPR	11	-0.0009
90	18	NUC	40	EALESPEH	8	
90	18	POL	718	ELLAACFA	8	
90	18	POL	718	ELLAACFAR	9	0.0002
85	17	POL	718	ELLAACFARSR	11	0.0062
95	19	NUC	43	ELLSFLPSDF	10	
95	19	NUC	43	ELLSFLPSDFF	11	
95	19	NUC	43	ESPEHCSPH	9	
95	19	NUC	43	ESPEHCSPHH	10	
95	19	POL	374	ESRLVVDF	8	
95	19	POL	374	ESRLVVDFSQF	11	
95	19	NUC	174	ETTVVRRR	8	0.0003
80	16	NUC	174	ETTVVRRRGR	10	0.0003
95	19	POL	631	FAAPFTQCGY	10	
85	17	POL	724	FARSRSGA	8	
80	16	POL	821	FASPLHVA	8	
80	16	POL	821	FASPLHVAWR	10	

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
90	18	ENV	13	FFPDHQLDPA	10	
85	17	ENV	13	FFPDHQLDPAF	11	
75	15	NUC	139	FGRETVLEY	9	
75	15	POL	244	FGVEPSGSGH	10	
95	19	NUC	122	FGVWIRTPPA	10	
95	19	NUC	122	FGVWIRTPPAY	11	
80	16	ENV	248	FILLLCLIF	9	
80	16	ENV	246	FLFILLLCLIF	11	
75	15	ENV	171	FLGPLLVLQA	10	
95	19	POL	513	FLLAQFTSA	9	0.0006
95	19	POL	562	FLLSLGIH	8	
95	19	ENV	256	FLLVLLDY	8	0.0050
100	20	POL	363	FLVDKNPH	8	
95	19	POL	658	FSPTYKAF	8	
95	19	X	63	FSSAGPCA	8	
90	18	X	63	FSSAGPCALR	10	
90	18	X	63	FSSAGPCALRF	11	
100	20	ENV	333	FSWLSLLVPF	10	0.0004
90	18	POL	536	FSYMDDVVLGA	11	
95	19	POL	656	FTFSPTYK	8	0.0100
95	19	POL	656	FTFSPTYKA	9	
95	19	POL	656	FTFSPTYKAF	10	0.0004
95	19	POL	635	FTQCGYPA	8	
95	19	POL	518	FTSAICSVVR	10	0.0003
95	19	POL	518	FTSAICSVVRR	11	0.0065
95	19	X	132	FVLGGCRH	8	
90	18	X	132	FVLGGCRHK	9	0.0430
90	18	POL	766	FVYVPSALNPA	11	
80	16	POL	754	GCAANWILR	9	
95	19	POL	630	GFAAPFTQCGY	11	
90	18	ENV	12	GFFPDHQLDPA	11	
75	15	ENV	170	GFLGPLLVLQA	11	
85	17	ENV	61	GGLLGWSPQA	10	
100	20	POL	360	GGVFLVDK	8	
100	20	POL	360	GGVFLVDKNPH	11	
75	15	POL	567	GIHLNPNK	8	
75	15	POL	567	GIHLNPNKTK	10	0.0025
75	15	POL	567	GIHLNPNKTKR	11	
85	17	POL	682	GLCQVFADA	9	0.0001
95	19	POL	627	GLLGFAAPF	9	0.0006
85	17	ENV	62	GLLGWSPQA	9	
95	19	X	57	GLPVCAFSSA	10	

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
95	19	POL	509	GLSPFLLA	8	
95	19	POL	509	GLSPFLLAQF	10	
85	17	NUC	29	GMDIDPYK	8	0.0006
85	17	NUC	29	GMDIDPYKEF	10	-0.0003
90	18	POL	735	GTDNSVVLSR	10	0.0010
90	18	POL	735	GTDNSVVLSRK	11	0.0140
80	16	POL	763	GTSFVYVPSA	10	
80	16	POL	245	GVEPSGSGH	9	
100	20	POL	361	GVFLVDKNPH	10	
80	16	POL	507	GVGLSPFLLA	10	
95	19	NUC	123	GVWIRTPPA	9	
95	19	NUC	123	GVWIRTPPAY	10	0.0047
95	19	NUC	123	GVWIRTPPAYR	11	0.1900
100	20	NUC	47	HCSPHHTA	8	
100	20	NUC	47	HCSPHHTALR	10	
80	16	POL	820	HFASPLHVA	9	
80	16	POL	820	HFASPLHVAWR	11	
95	19	X	49	HGAHLSLR	8	
85	17	ENV	60	HGGLLGWSPQA	11	
90	18	NUC	104	HISCLTFGR	9	-0
75	15	POL	569	HLNPNKTK	8	
75	15	POL	569	HLNPNKTKR	9	
90	18	X	52	HLSLRGLPVCA	11	
80	16	POL	491	HLYSHPIILGF	11	
85	17	POL	715	HTAELLAA	8	
85	17	POL	715	HTAELLAACF	10	
85	17	POL	715	HTAELLAACFA	11	
100	20	POL	149	HTLWKAGILY	10	0.0440
100	20	POL	149	HTLWKAGILYK	11	0.5400
95	19	POL	522	ICSVVRRA	8	
95	19	POL	522	ICSVVRRAF	9	
95	19	POL	522	ICSVVRRAFPH	11	
90	18	NUC	32	IDPYKEFGA	9	
90	18	POL	617	IDWKVQQR	8	
100	20	ENV	381	IFFCLWVY	8	
95	19	ENV	255	IFLLVLLDY	9	
80	16	POL	734	IGTDNSVVLSR	11	
100	20	ENV	249	ILLLCLIF	8	
80	16	POL	760	ILRGTSFVY	9	0.0440
90	18	NUC	105	ISCLTFGR	8	0.0004
90	18	POL	625	IVGLLGFA	8	
90	18	POL	625	IVGLLGFAA	9	

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
90	18	POL	625	IVGLLGFAAPF	11	
100	20	POL	153	KAGILYKR	8	0.0002
80	16	POL	503	KIPMGVGLSPF	11	
75	15	POL	108	KLIMPARF	8	
75	15	POL	108	KLIMPARFY	9	
80	16	POL	610	KLPVNRPIDWK	11	
85	17	POL	574	KTKRWGYSLNF	11	
75	15	X	130	KVFVLGGCR	9	0.0420
75	15	X	130	KVFVLGGCRH	10	
95	19	POL	55	KVGNFTGLY	9	0.2100
85	17	POL	720	LAACFARSR	9	0.0058
95	19	X	16	LCLRPVGA	8	
90	18	X	16	LCLRPVGAESR	11	
95	19	POL	683	LCQVFADA	8	
100	20	POL	125	LDKGIKPY	8	
100	20	POL	125	LDKGIKPYY	9	
80	16	X	9	LDPARDVLCLR	11	
95	19	ENV	195	LDSWWTSLNF	10	
85	17	NUC	31	LDTASALY	8	
85	17	NUC	31	LDTASALYR	9	0.0004
80	16	NUC	31	LDTASALYREA	11	
95	19	POL	417	LDVSAAFY	8	
95	19	POL	417	LDVSAAFYH	9	
80	16	ENV	247	LFILLLCLIF	10	
95	19	POL	544	LGAKSVQH	8	
80	16	POL	753	LGCAANWILR	10	
75	15	POL	566	LGIHLNPNK	9	
75	15	POL	566	LGIHLNPNKTK	11	
95	19	ENV	172	LGPLLVLQA	9	
95	19	ENV	172	LGPLLVLQAGF	11	
95	19	ENV	254	LIFLLVLLDY	10	0.0022
100	20	POL	109	LIMPARFY	8	-0.0002
90	18	POL	719	LLAACFAR	8	0.0024
85	17	POL	719	LLAACFARSR	10	
95	19	POL	514	LLAQFTSA	8	
85	17	NUC	30	LLDTASALY	9	0.0013
85	17	NUC	30	LLDTASALYR	10	0.0050
80	16	POL	752	LLGCAANWILR	11	
95	19	POL	628	LLGFAAPF	8	
85	17	ENV	63	LLGWSPQA	8	
100	20	ENV	378	LLPIFFCLWVY	11	0.0230
95	19	NUC	44	LLSFLPSDF	9	

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
95	19	NUC	44	LLSFLPSDFF	10	
95	19	ENV	175	LLVLQAGF	8	
95	19	ENV	175	LLVLQAGFF	9	0.0006
100	20	ENV	338	LLVPFVQWF	9	
85	17	NUC	100	LLWFHISCLTF	11	
95	19	NUC	45	LSFLPSDF	8	
95	19	NUC	45	LSFLPSDFF	9	0.0006
95	19	POL	415	LSLDVSAA	8	
95	19	POL	415	LSLDVSAAF	9	
95	19	POL	415	LSLDVSAAFY	10	0.0004
95	19	POL	415	LSLDVSAAFYH	11	
75	15	POL	564	LSLGIHLNPNK	11	
100	20	ENV	336	LSLLVPFVQWF	11	
95	19	X	53	LSLRGLPVCA	10	
95	19	X	53	LSLRGLPVCAF	11	
95	19	POL	510	LSPFLLAQF	9	
85	17	POL	742	LSRKYTSF	8	
95	19	NUC	169	LSTLPETTVVR	11	-0.0009
75	15	ENV	16	LSVPNPLGF	9	
100	20	POL	412	LSWLSLDVSA	10	0.0048
95	19	POL	412	LSWLSLDVSAA	11	
75	15	POL	3	LSYQHFRK	8	
75	15	NUC	137	LTFGRETVLEY	11	
85	17	POL	99	LTVNEKRR	8	-0.0002
95	19	ENV	176	LVLQAGFF	8	
100	20	ENV	339	LVPFVQWF	8	
90	18	NUC	119	LVSFGVWIR	9	0.0028
100	20	POL	377	LVVDFSQF	8	
100	20	POL	377	LVVDFSQFSR	10	0.0016
95	19	ENV	238	MCLRRFIIF	9	
75	15	ENV	238	MCLRRFIIFLF	11	
90	18	POL	539	MDDVVLGA	8	
90	18	POL	539	MDDVVLGAK	9	
90	18	NUC	30	MDIDPYKEF	9	
90	18	NUC	30	MDIDPYKEFGA	11	
80	16	POL	506	MGVGLSPF	8	
80	16	POL	506	MGVGLSPFLLA	11	
85	17	ENV	360	MMWYWGPSLY	10	0.0500
80	16	X	103	MSTTDLEA	8	
75	15	X	103	MSTTDLEAY	9	0.0008
75	15	X	103	MSTTDLEAYF	10	
75	15	X	103	MSTTDLEAYFK	11	

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
95	19	POL	561	NFLLSLGIH	9	
90	18	NUC	75	NLEDPASR	8	-0.0002
95	19	POL	45	NLNVSIPWTH	10	
95	19	POL	45	NLNVSIPWTHK	11	-0.0009
75	15	ENV	15	NLSVPNPLGF	10	
90	18	POL	411	NLSWLSLDVSA	11	
75	15	ENV	215	NSQSPTSNH	9	
90	18	POL	738	NSVVLSRK	8	0.0006
90	18	POL	738	NSVVLSRKY	9	0.0002
100	20	POL	47	NVSIPWTH	8	
100	20	POL	47	NVSIPWTHK	9	0.0820
90	18	POL	775	PADDPSRGR	9	0.0008
95	19	POL	641	PALMPLYA	8	
75	15	X	145	PAPCNFFTSA	10	
80	16	X	11	PARDVLCLR	9	0.0002
90	18	POL	355	PARVTGGVF	9	
75	15	ENV	83	PASTNRQSGR	10	
95	19	NUC	130	PAYRPPNA	8	
90	18	X	68	PCALRFTSA	9	
85	17	X	68	PCALRFTSAR	10	
75	15	X	147	PCNFFTSA	8	
95	19	ENV	15	PDHQLDPA	8	
90	18	ENV	15	PDHQLDPAF	9	
95	19	POL	512	PFLLAQFTSA	10	
95	19	POL	634	PFTQCGYPA	9	
100	20	ENV	233	PGYRWMCLR	9	0.0008
95	19	ENV	233	PGYRWMCLRR	10	0.0048
95	19	ENV	233	PGYRWMCLRRF	11	
90	18	POL	616	PIDWKVCQR	9	0.0002
100	20	ENV	380	PIFFCLWVY	9	0.0011
85	17	POL	713	PIHTAELLA	9	
85	17	POL	713	PIHTAELLAA	10	
80	16	POL	496	PILGFRK	8	
100	20	ENV	314	PIPSSWAF	8	
80	16	ENV	314	PIPSSWAFA	9	
100	20	POL	124	PLDKGIKPY	9	0.0001
100	20	POL	124	PLDKGIKPYY	10	0.0002
95	19	POL	20	PLEEELPR	8	0.0002
90	18	POL	20	PLEEELPRLA	10	
95	19	ENV	10	PLGFFPDH	8	
100	20	POL	427	PLHPAAMPH	9	0.0012
95	19	ENV	174	PLLVLQAGF	9	

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
95	19	ENV	174	PLLVLQAGFF	10	
80	16	POL	711	PLPIHTAELLA	11	
100	20	POL	2	PLSYQHFR	8	-0.0002
75	15	POL	2	PLSYQHFRK	9	0.0011
85	17	POL	98	PLTVNEKR	8	0.0002
85	17	POL	98	PLTVNEKRR	9	0.0008
80	16	POL	505	PMGVGLSPF	9	
85	17	POL	797	PTTGRTSLY	9	0.0001
85	17	POL	797	PTTGRTSLYA	10	
95	19	X	59	PVCAFSSA	8	
90	18	X	20	PVGAESRGR	9	0.0002
85	17	POL	612	PVNRPIDWK	9	0.0310
95	19	POL	654	QAFTFSPTY	9	0.0030
95	19	POL	654	QAFTFSPTYK	10	0.0450
95	19	POL	654	QAFTFSPTYKA	11	
80	16	ENV	179	QAGFFLLTR	9	
80	16	ENV	107	QAMQWNSTTF	10	
80	16	ENV	107	QAMQWNSTTFH	11	
95	19	POL	637	QCGYPALMPLY	11	
95	19	POL	517	QFTSAICSVVR	11	
75	15	NUC	169	QSPRRRRSQSR	11	
80	16	POL	189	QSSGILSR	8	
95	19	POL	528	RAFPHCLA	8	
95	19	POL	528	RAFPHCLAF	9	0.0015
95	19	POL	528	RAFPHCLAFSY	11	0.1200
85	17	NUC	28	RDLLDTASA	9	
85	17	NUC	28	RDLLDTASALY	11	
95	19	X	13	RDVLCLRPVGA	11	
100	20	ENV	332	RFSWLSLLVPF	11	
95	19	X	56	RGLPVCAF	8	
95	19	X	56	RGLPVCAFSSA	11	
100	20	NUC	152	RGRSPRRR	8	
80	16	POL	762	RGTSFVYVPSA	11	
90	18	POL	624	RIVGLLGF	8	
90	18	POL	624	RIVGLLGFA	9	
90	18	POL	624	RIVGLLGFAA	10	
75	15	POL	106	RLKLIMPA	8	
75	15	POL	106	RLKLIMPAR	9	0.0950
75	15	POL	106	RLKLIMPARF	10	
75	15	POL	106	RLKLIMPARFY	11	
75	15	X	128	RLKVFVLGGCR	11	
95	19	POL	376	RLVVDFSQF	9	0.0006

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
95	19	POL	376	RLVVDFSQFSR	11	0.2800
95	19	NUC	183	RSPRRRTPSPR	11	-0.0007
75	15	NUC	167	RSQSPRRR	8	
75	15	NUC	167	RSQSPRRRR	9	
90	18	POL	353	RTPARVTGGVF	11	
95	19	NUC	127	RTPPAYRPPNA	11	
95	19	NUC	188	RTPSPRRR	8	-0.0002
95	19	NUC	188	RTPSPRRRR	9	0.0054
80	16	POL	818	RVHFASPLH	9	
75	15	POL	818	RVHFASPLHVA	11	
100	20	POL	357	RVTGGVFLVDK	11	0.0190
90	18	X	65	SAGPCALR	8	-0.0002
90	18	X	65	SAGPCALRF	9	-0.0003
95	19	POL	520	SAICSVVR	8	-0.0002
95	19	POL	520	SAICSVVRR	9	0.0058
95	19	POL	520	SAICSVVRRA	10	
95	19	POL	520	SAICSVVRRAF	11	
90	18	POL	771	SALNPADDPSR	11	-0.0004
100	20	POL	165	SASFCGSPY	9	
90	18	NUC	121	SFGVWIRTPPA	11	
95	19	NUC	46	SFLPSDFF	8	
75	15	POL	748	SFPWLLGCA	9	
75	15	POL	748	SFPWLLGCAA	10	
80	16	POL	765	SFVYVPSA	8	
100	20	POL	49	SIPWTHKVGNF	11	
95	19	ENV	194	SLDSWWTSLNF	11	
95	19	POL	416	SLDVSAAF	8	
95	19	POL	416	SLDVSAAFY	9	0.0016
95	19	POL	416	SLDVSAAFYH	10	
75	15	POL	565	SLGIHLNPNK	10	
100	20	ENV	337	SLLVPFVQWF	10	
95	19	X	54	SLRGLPVCA	9	
95	19	X	54	SLRGLPVCAF	10	0.0004
90	18	X	64	SSAGPCALR	9	0.0080
90	18	X	64	SSAGPCALRF	10	-0.0003
95	19	NUC	170	STLPETTVVR	10	0.0007
95	19	NUC	170	STLPETTVVRR	11	0.0150
80	16	ENV	85	STNRQSGR	8	
75	15	X	104	STTDLEAY	8	
75	15	X	104	STTDLEAYF	9	
75	15	X	104	STTDLEAYFK	10	0.0066
75	15	ENV	17	SVPNPLGF	8	

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
90	18	POL	739	SVVLSRKY	8	-0.0002
85	17	POL	739	SVVLSRKYTSF	11	
95	19	POL	524	SVVRRAFPH	9	0.1100
85	17	POL	716	TAELLAACF	9	
85	17	POL	716	TAELLAACFA	10	
85	17	POL	716	TAELLAACFAR	11	0.0006
80	16	NUC	33	TASALYREA	9	
100	20	ENV	311	TCIPIPSSWA	10	
100	20	ENV	311	TCIPIPSSWAF	11	
80	16	X	106	TDLEAYFK	8	
90	18	POL	736	TDNSVVLSR	9	
90	18	POL	736	TDNSVVLSRK	10	0.0006
90	18	POL	736	TDNSVVLSRKY	11	
75	15	NUC	138	TFGRETVLEY	10	
95	19	POL	657	TFSPTYKA	8	
95	19	POL	657	TFSPTYKAF	9	
100	20	POL	359	TGGVFLVDK	9	0.0007
85	17	POL	799	TGRTSLYA	8	
95	19	NUC	171	TLPETTVVR	9	0.0008
95	19	NUC	171	TLPETTVVRR	10	0.0007
95	19	NUC	171	TLPETTVVRRR	11	0.0005
100	20	POL	150	TLWKAGILY	9	0.1300
100	20	POL	150	TLWKAGILYK	10	5.3000
100	20	POL	150	TLWKAGILYKR	11	0.0082
95	19	POL	519	TSAICSVVR	9	0.0005
95	19	POL	519	TSAICSVVRR	10	0.0018
95	19	POL	519	TSAICSVVRRA	11	
75	15	POL	747	TSFPWLLGCA	10	
75	15	POL	747	TSFPWLLGCAA	11	
80	16	POL	764	TSFVYVPSA	9	
75	15	X	105	TTDLEAYF	8	
75	15	X	105	TTDLEAYFK	9	0.0006
85	17	POL	798	TTGRTSLY	8	0.0004
85	17	POL	798	TTGRTSLYA	9	
75	15	ENV	278	TTSTGPCK	8	
80	16	NUC	175	TTVVRRRGR	9	0.0008
80	16	NUC	176	TVVRRRGR	8	0.0003
80	16	NUC	176	TVVRRRGRSPR	11	
95	19	X	60	VCAFSSAGPCA	11	
85	17	POL	621	VCQRIVGLLGF	11	
100	20	POL	379	VDFSQFSR	8	
100	20	POL	362	VFLVDKNPH	9	

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
80	16	X	131	VFVLGGCR	8	
80	16	X	131	VFVLGGCRH	9	
75	15	X	131	VFVLGGCRHK	10	
95	19	X	21	VGAESRGR	8	
95	19	POL	626	VGLLGFAA	8	
95	19	POL	626	VGLLGFAAPF	10	
80	16	POL	508	VGLSPFLLA	9	
80	16	POL	508	VGLSPFLLAQF	11	
95	19	POL	56	VGNFTGLY	8	
85	17	POL	96	VGPLTVNEK	9	0.0007
85	17	POL	96	VGPLTVNEKR	10	
85	17	POL	96	VGPLTVNEKRR	11	
95	19	X	15	VLCLRPVGA	9	
95	19	POL	543	VLGAKSVQH	9	
90	18	X	133	VLGGCRHK	8	0.0150
80	16	ENV	177	VLQAGFFLLTR	11	
85	17	POL	741	VLSRKYTSF	9	
90	18	NUC	120	VSFGVWIR	8	0.0040
100	20	POL	48	VSIPWTHK	8	0.0130
100	20	POL	358	VTGGVFLVDK	10	0.0390
100	20	POL	378	VVDFSQFSR	9	0.0015
90	18	POL	542	VVLGAKSVQH	10	
85	17	POL	740	VVLSRKYTSF	10	0.0004
95	19	POL	525	VVRRAFPH	8	
95	19	POL	525	VVRRAFPHCLA	11	
80	16	NUC	177	VVRRRGRSPR	10	0.0027
80	16	NUC	177	VVRRRGRSPRR	11	
90	18	NUC	102	WFHISCLTF	9	
90	18	NUC	102	WFHISCLTFGR	11	
85	17	NUC	28	WGMDIDPY	8	
85	17	NUC	28	WGMDIDPYK	9	-0.0003
85	17	NUC	28	WGMDIDPYKEF	11	
85	17	POL	578	WGYSLNFMGY	10	
80	16	POL	759	WILRGTSF	8	
80	16	POL	759	WILRGTSFVY	10	0.0076
95	19	NUC	125	WIRTPPAY	8	-0.0002
95	19	NUC	125	WIRTPPAYR	9	0.0008
90	18	POL	314	WLQFRNSK	8	-0.0002
100	20	POL	414	WLSLDVSA	8	
95	19	POL	414	WLSLDVSAA	9	
95	19	POL	414	WLSLDVSAAF	10	
95	19	POL	414	WLSLDVSAAFY	11	0.0034

HBV A03 Motif With Binding Data

Conservancy	Freq.	Protein	Position	Sequence	AA	A*0301
100	20	ENV	335	WLSLLVPF	8	
85	17	NUC	26	WLWGMDIDPY	10	0.0002
85	17	NUC	26	WLWGMDIDPYK	11	0.0030
95	19	ENV	237	WMCLRRFIIF	10	0.0004
85	17	ENV	359	WMMWYWGPSLY	11	0.0009
100	20	POL	52	WTHKVGNF	8	
100	20	POL	147	YLHTLWKA	8	
100	20	POL	122	YLPLDKGIK	9	0.0001
100	20	POL	122	YLPLDKGIKPY	11	-0.0004
90	18	NUC	118	YLVSFGVWIR	10	0.0005
90	18	POL	538	YMDDVVLGA	9	0.0001
90	18	POL	538	YMDDVVLGAK	10	0.0330
80	16	POL	493	YSHPIILGF	9	
80	16	POL	493	YSHPIILGFR	10	
80	16	POL	493	YSHPIILGFRK	11	
85	17	POL	580	YSLNFMGY	8	-0.0002
75	15	POL	746	YTSFPWLLGCA	11	
90	18	POL	768	YVPSALNPA	9	

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Table XVII

A11 Motif With Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*1101
85	17	POL	721	AACFARSR	8	
95	19	POL	632	AAPFTQCGY	9	
90	18	POL	776	ADDPSRGR	8	
95	19	POL	529	AFPHCLAFSY	10	
90	18	X	62	AFSSAGPCALR	11	
95	19	POL	655	AFTFSPTY	8	
95	19	POL	655	AFTFSPTYK	9	
80	16	ENV	180	AGFFLLTR	8	
95	19	POL	18	AGPLEEELPR	10	
95	19	POL	521	AICSVVRR	8	
95	19	NUC	41	ALESPEHCSPH	11	
90	18	POL	772	ALNPADDPSR	10	
85	17	X	70	ALRFTSAR	8	
80	16	ENV	108	AMQWNSTTFH	10	
100	20	POL	166	ASFCGSPY	8	
80	16	POL	822	ASPLHVAWR	9	
75	15	ENV	84	ASTNRQSGR	9	
80	16	POL	755	CAANWILR	8	
85	17	X	69	CALRFTSAR	9	
80	16	X	6	CCQLDPAR	8	
75	15	POL	607	CFRKLPVNR	9	
95	19	POL	638	CGYPALMPLY	10	
95	19	ENV	253	CLIFLLVLLDY	11	
90	18	X	17	CLRPVGAESR	10	
100	20	NUC	48	CSPHHTALR	9	
95	19	POL	523	CSVVRRAFPH	10	
90	18	POL	540	DDVVLGAK	8	
85	17	NUC	29	DLLDTASALY	10	
85	17	NUC	29	DLLDTASALYR	11	
90	18	POL	737	DNSVVLSR	8	
90	18	POL	737	DNSVVLSRK	9	
90	18	POL	737	DNSVVLSRKY	10	
85	17	NUC	32	DTASALYR	8	
95	19	POL	418	DVSAAFYH	8	
90	18	POL	541	DVVLGAKSVQH	11	
95	19	POL	17	EAGPLEEELPR	11	
90	18	NUC	40	EALESPEH	8	
90	18	POL	718	ELLAACFAR	9	
85	17	POL	718	ELLAACFARSR	11	
95	19	NUC	43	ESPEHCSPH	9	
95	19	NUC	43	ESPEHCSPHH	10	
95	19	NUC	174	ETTVVRRR	8	

A11 Motif With Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*1101
80	16	NUC	174	ETTVVRRRGR	10	
95	19	POL	631	FAAPFTQCGY	10	
80	16	POL	821	FASPLHVAWR	10	
75	15	NUC	139	FGRETVLEY	9	
75	15	POL	244	FGVEPSGSGH	10	
95	19	NUC	122	FGVWIRTPPAY	11	
95	19	POL	562	FLLSLGIH	8	
95	19	ENV	256	FLLVLLDY	8	
100	20	POL	363	FLVDKNPH	8	
90	18	X	63	FSSAGPCALR	10	
95	19	POL	656	FTFSPTYK	8	
95	19	POL	518	FTSAICSVVR	10	
95	19	POL	518	FTSAICSVVRR	11	
95	19	X	132	FVLGGCRH	8	
90	18	X	132	FVLGGCRHK	9	
80	16	POL	754	GCAANWILR	9	
95	19	POL	630	GFAAPFTQCGY	11	
100	20	POL	360	GGVFLVDK	8	
100	20	POL	360	GGVFLVDKNPH	11	
75	15	POL	567	GIHLNPNK	8	
75	15	POL	567	GIHLNPNKTK	10	
75	15	POL	567	GIHLNPNKTKR	11	
85	17	NUC	29	GMDIDPYK	8	
95	19	POL	44	GNLNVSIPWTH	11	
90	18	POL	735	GTDNSVVLSR	10	
90	18	POL	735	GTDNSVVLSRK	11	
80	16	POL	245	GVEPSGSGH	9	
100	20	POL	361	GVFLVDKNPH	10	
95	19	NUC	123	GVWIRTPPAY	10	
95	19	NUC	123	GVWIRTPPAYR	11	
100	20	NUC	47	HCSPHHTALR	10	
80	16	POL	820	HFASPLHVAWR	11	
95	19	X	49	HGAHLSLR	8	
90	18	NUC	104	HISCLTFGR	9	
75	15	POL	569	HLNPNKTK	8	
75	15	POL	569	HLNPNKTKR	9	
100	20	POL	149	HTLWKAGILY	10	
100	20	POL	149	HTLWKAGILYK	11	
95	19	POL	522	ICSVVRRAFPH	11	
90	18	POL	617	IDWKVCQR	8	
100	20	ENV	381	IFFCLWVY	8	
95	19	ENV	255	IFLLVLLDY	9	

A11 Motif With Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*1101
80	16	POL	734	IGTDNSVVLSR	11	
80	16	POL	760	ILRGTSFVY	9	
90	18	NUC	105	ISCLTFGR	8	
100	20	POL	153	KAGILYKR	8	
75	15	POL	108	KLIMPARFY	9	
80	16	POL	610	KLPVNRPIDWK	11	
75	15	X	130	KVFVLGGCR	9	
75	15	X	130	KVFVLGGCRH	10	
95	19	POL	55	KVGNFTGLY	9	
85	17	POL	720	LAACFARSR	9	
90	18	X	16	LCLRPVGAESR	11	
100	20	POL	125	LDKGIKPY	8	
100	20	POL	125	LDKGIKPYY	9	
80	16	X	9	LDPARDVLCLR	11	
85	17	NUC	31	LDTASALY	8	
85	17	NUC	31	LDTASALYR	9	
95	19	POL	417	LDVSAAFY	8	
95	19	POL	417	LDVSAAFYH	9	
95	19	POL	544	LGAKSVQH	8	
80	16	POL	753	LGCAANWILR	10	
75	15	POL	566	LGIHLNPNK	9	
75	15	POL	566	LGIHLNPNKTK	11	
95	19	ENV	254	LIFLLVLLDY	10	
100	20	POL	109	LIMPARFY	8	
90	18	POL	719	LLAACFAR	8	
85	17	POL	719	LLAACFARSR	10	
85	17	NUC	30	LLDTASALY	9	
85	17	NUC	30	LLDTASALYR	10	
80	16	POL	752	LLGCAANWILR	11	
100	20	ENV	378	LLPIFFCLWVY	11	
90	18	POL	773	LNPADDPSR	9	
90	18	POL	773	LNPADDPSRGR	11	
75	15	POL	570	LNPNKTKR	8	
75	15	POL	570	LNPNKTKRWGY	11	
95	19	POL	46	LNVSIPWTH	9	
95	19	POL	46	LNVSIPWTHK	10	
95	19	POL	415	LSLDVSAAFY	10	
95	19	POL	415	LSLDVSAAFYH	11	
75	15	POL	564	LSLGIHLNPNK	11	
95	19	NUC	169	LSTLPETTVVR	11	
75	15	POL	3	LSYQHFRK	8	
75	15	NUC	137	LTFGRETVLEY	11	

A11 Motif With Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*1101
85	17	POL	99	LTVNEKRR	8	
90	18	NUC	119	LVSFGVWIR	9	
100	20	POL	377	LVVDFSQFSR	10	
90	18	POL	539	MDDVVLGAK	9	
85	17	ENV	360	MMWYWGPSLY	10	
75	15	X	103	MSTTDLEAY	9	
75	15	X	103	MSTTDLEAYFK	11	
95	19	POL	561	NFLLSLGIH	9	
90	18	NUC	75	NLEDPASR	8	
95	19	POL	45	NLNVSIPWTH	10	
95	19	POL	45	NLNVSIPWTHK	11	
75	15	ENV	215	NSQSPTSNH	9	
90	18	POL	738	NSVVLSRK	8	
90	18	POL	738	NSVVLSRKY	9	
100	20	POL	47	NVSIPWTH	8	
100	20	POL	47	NVSIPWTHK	9	
90	18	POL	775	PADDPSRGR	9	
80	16	X	11	PARDVLCLR	9	
75	15	ENV	83	PASTNRQSGR	10	
85	17	X	68	PCALRFTSAR	10	
100	20	ENV	233	PGYRWMCLR	9	
95	19	ENV	233	PGYRWMCLRR	10	
90	18	POL	616	PIDWKVCQR	9	
100	20	ENV	380	PIFFCLWVY	9	
80	16	POL	496	PILGFRK	8	
100	20	POL	124	PLDKGIKPY	9	
100	20	POL	124	PLDKGIKPYY	10	
95	19	POL	20	PLEEELPR	8	
95	19	ENV	10	PLGFFPDH	8	
100	20	POL	427	PLHPAAMPH	9	
100	20	POL	2	PLSYQHFR	8	
75	15	POL	2	PLSYQHFRK	9	
85	17	POL	98	PLTVNEKR	8	
85	17	POL	98	PLTVNEKRR	9	
75	15	POL	572	PNKTKRWGY	9	
85	17	POL	797	PTTGRTSLY	9	
90	18	X	20	PVGAESRGR	9	
85	17	POL	612	PVNRPIDWK	9	
95	19	POL	654	QAFTFSPTY	9	
95	19	POL	654	QAFTFSPTYK	10	
80	16	ENV	179	QAGFFLLTR	9	
80	16	ENV	107	QAMQWNSTTFH	11	

A11 Motif With Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*1101
95	19	POL	637	QCGYPALMPLY	11	
95	19	POL	517	QFTSAICSVVR	11	
75	15	NUC	169	QSPRRRRSQSR	11	
80	16	POL	189	QSSGILSR	8	
95	19	POL	528	RAFPHCLAFSY	11	
85	17	NUC	28	RDLLDTASALY	11	
100	20	NUC	152	RGRSPRRR	8	
75	15	POL	106	RLKLIMPAR	9	
75	15	POL	106	RLKLIMPARFY	11	
75	15	X	128	RLKVFVLGGCR	11	
95	19	POL	376	RLVVDFSQFSR	11	3
95	19	NUC	183	RSPRRRTPSPR	11	
75	15	NUC	167	RSQSPRRR	8	
75	15	NUC	167	RSQSPRRRR	9	
95	19	NUC	188	RTPSPRRR	8	
95	19	NUC	188	RTPSPRRRR	9	
80	16	POL	818	RVHFASPLH	9	
100	20	POL	357	RVTGGVFLVDK	11	
90	18	X	65	SAGPCALR	8	
95	19	POL	520	SAICSVVR	8	
95	19	POL	520	SAICSVVRR	9	
90	18	POL	771	SALNPADDPSR	11	
100	20	POL	165	SASFCGSPY	9	
95	19	POL	416	SLDVSAAFY	9	
95	19	POL	416	SLDVSAAFYH	10	
75	15	POL	565	SLGIHLNPNK	10	
90	18	X	64	SSAGPCALR	9	
95	19	NUC	170	STLPETTVVR	10	
95	19	NUC	170	STLPETTVVRR	11	1
80	16	ENV	85	STNRQSGR	8	
75	15	X	104	STTDLEAY	8	
75	15	X	104	STTDLEAYFK	10	2
90	18	POL	739	SVVLSRKY	8	
95	19	POL	524	SVVRRAFPH	9	
85	17	POL	716	TAELLAACFAR	11	
80	16	X	106	TDLEAYFK	8	
90	18	POL	736	TDNSVVLSR	9	
90	18	POL	736	TDNSVVLSRK	10	
90	18	POL	736	TDNSVVLSRKY	11	
75	15	NUC	138	TFGRETVLEY	10	
100	20	POL	359	TGGVFLVDK	9	
95	19	NUC	171	TLPETTVVR	9	

A11 Motif With Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*1101
95	19	NUC	171	TLPETTVVRR	10	
95	19	NUC	171	TLPETTVVRRR	11	
100	20	POL	150	TLWKAGILY	9	
100	20	POL	150	TLWKAGILYK	10	
100	20	POL	150	TLWKAGILYKR	11	
95	19	POL	560	TNFLLSLGIH	10	
95	19	POL	519	TSAICSVVR	9	
95	19	POL	519	TSAICSVVRR	10	
75	15	X	105	TTDLEAYFK	9	
85	17	POL	798	TTGRTSLY	8	
75	15	ENV	278	TTSTGPCK	8	
80	16	NUC	175	TTVVRRRGR	9	
80	16	NUC	176	TVVRRRGR	8	
80	16	NUC	176	TVVRRRGRSPR	11	
100	20	POL	379	VDFSQFSR	8	
100	20	POL	362	VFLVDKNPH	9	
80	16	X	131	VFVLGGCR	8	
80	16	X	131	VFVLGGCRH	9	
75	15	X	131	VFVLGGCRHK	10	
95	19	X	21	VGAESRGR	8	
95	19	POL	56	VGNFTGLY	8	
85	17	POL	96	VGPLTVNEK	9	
85	17	POL	96	VGPLTVNEKR	10	
85	17	POL	96	VGPLTVNEKRR	11	
95	19	POL	543	VLGAKSVQH	9	
90	18	X	133	VLGGCRHK	8	
80	16	ENV	177	VLQAGFFLLTR	11	
85	17	POL	613	VNRPIDWK	8	
90	18	NUC	120	VSFGVWIR	8	
100	20	POL	48	VSIPWTHK	8	
100	20	POL	358	VTGGVFLVDK	10	
100	20	POL	378	VVDFSQFSR	9	
90	18	POL	542	VVLGAKSVQH	10	
95	19	POL	525	VVRNAFPH	8	
80	16	NUC	177	VVRRRGRSPR	10	
80	16	NUC	177	VVRRRGRSPRR	11	
90	18	NUC	102	WFHISCLTFGR	11	
85	17	NUC	28	WGMDIDPY	8	
85	17	NUC	28	WGMDIDPYK	9	
85	17	POL	578	WGYSLNFMGY	10	
80	16	POL	759	WILRGTSFVY	10	
95	19	NUC	125	WIRTPPAY	8	

A11 Motif With Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*1101
95	19	NUC	125	WIRTPPAYR	9	
90	18	POL	314	WLQFRNSK	8	
95	19	POL	414	WLSLDVSAAFY	11	
85	17	NUC	26	WLWGMDIDPY	10	
85	17	NUC	26	WLWGMDIDPYK	11	
85	17	ENV	359	WMMWYWGPSLY	11	
100	20	POL	122	YLPLDKGIK	9	
100	20	POL	122	YLPLDKGIKPY	11	
90	18	NUC	118	YLVSFGVWIR	10	
90	18	POL	538	YMDDVVLGAK	10	
80	16	POL	493	YSHPIILGFR	10	
80	16	POL	493	YSHPIILGFRK	11	
85	17	POL	580	YSLNFMGY	8	
				265		

Table XVIII

HBV A24 Motif With Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*2401
95	19	POL	529	AFPHCLAF	8	
95	19	X	62	AFSSAGPCAL	10	0.0012
90	18	POL	535	AFSYMDDVVL	10	0.0009
95	19	POL	655	AFTFSPTYKAF	11	
80	16	ENV	108	AMQWNSTTF	9	
100	20	NUC	131	AYRPPNAPI	9	0.0310
100	20	NUC	131	AYRPPNAPIL	10	0.0042
75	15	POL	607	CFRKLPVNRPI	11	
85	17	POL	618	DWKVCQRI	8	
85	17	POL	618	DWKVCQRIVGL	11	
90	18	ENV	262	DYQGMLPVCPL	11	0.0002
90	18	NUC	117	EYLVSFGVW	9	
90	18	NUC	117	EYLVSFGVWI	10	
100	20	ENV	382	FFCLWVYI	8	
80	16	ENV	182	FFLLTRIL	8	
80	16	ENV	182	FFLLTRILTI	10	
85	17	ENV	13	FFPDHQLDPAF	11	
80	16	ENV	181	GFFLLTRI	8	
80	16	ENV	181	GFFLLTRIL	9	
80	16	ENV	181	GFFLLTRILTI	11	
95	19	ENV	12	GFFPDHQL	8	
75	15	ENV	170	GFLGPLLVL	9	
80	16	POL	500	GFRKIPMGVGL	11	
85	17	NUC	29	GMDIDPYKEF	10	
90	18	ENV	265	GMLPVCPL	8	
85	17	NUC	25	GWLWGMDI	8	
85	17	ENV	65	GWSPQAQGI	9	0.0024
85	17	ENV	65	GWSPQAQGIL	10	0.0003
95	19	POL	639	GYPALMPL	8	
95	19	ENV	234	GYRWMCLRRF	10	0.0007
95	19	ENV	234	GYRWMCLRRFI	11	
75	15	POL	579	GYSLNFMGYVI	11	
80	16	POL	820	HFASPLHVAW	10	
75	15	POL	7	HFRKLLLL	8	
100	20	POL	146	HYLHTLWKAGI	11	
100	20	ENV	381	IFFCLWVYI	9	0.0087
80	16	ENV	245	IFLFILLL	8	
80	16	ENV	245	IFLFILLLCL	10	
80	16	ENV	245	IFLFILLLCLI	11	
85	17	ENV	358	IWMMWYWGPSL	11	0.0004
95	19	POL	395	KFAVPNLQSL	10	0.0020
100	20	POL	121	KYLPLDKGI	9	

HBV A24 Motif With Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*2401
85	17	POL	745	KYTSFPWL	8	
85	17	POL	745	KYTSFPWLL	9	5.3000
80	16	ENV	247	LFILLLCL	8	
80	16	ENV	247	LFILLLCLI	9	
80	16	ENV	247	LFILLLCLIF	10	
80	16	ENV	247	LFILLLCLIFL	11	
95	19	POL	643	LMPLYACI	8	
90	18	NUC	101	LWFHISCL	8	
85	17	NUC	101	LWFHISCLTF	10	
80	16	POL	492	LYSHPIIL	8	
80	16	POL	492	LYSHPIILGF	10	1.1000
85	17	ENV	360	MMWYWGPSL	9	0.0060
85	17	ENV	361	MWYWGPSL	8	0.0005
95	19	POL	561	NFLLSLGI	8	
95	19	POL	561	NFLLSLGIHL	10	0.0099
80	16	POL	758	NWILRGTSF	9	
95	19	POL	512	PFLLAQFTSAI	11	
95	19	POL	634	PFTQCGYPAL	10	0.0002
95	19	ENV	341	PFVQWFVGL	9	0.0003
80	16	POL	505	PMGVGLSPF	9	
80	16	POL	505	PMGVGLSPFL	10	
80	16	POL	505	PMGVGLSPFLL	11	
80	16	POL	750	PWLLGCAANW	10	
80	16	POL	750	PWLLGCAANWI	11	
100	20	POL	51	PWTHKVGNF	9	0.0290
95	19	ENV	344	QWFVGLSPTVW	11	
75	15	ENV	242	RFIIFLFI	8	
75	15	ENV	242	RFIIFLFIL	9	
75	15	ENV	242	RFIIFLFILL	10	
75	15	ENV	242	RFIIFLFILLL	11	
100	20	ENV	332	RFSWLSLL	8	
100	20	ENV	332	RFSWLSLLVPF	11	
85	17	POL	577	RWGYSLNF	8	
95	19	ENV	236	RWMCLRRF	8	
95	19	ENV	236	RWMCLRRFI	9	0.0710
95	19	ENV	236	RWMCLRRFII	10	1.1000
95	19	ENV	236	RWMCLRRFIIF	11	
100	20	POL	167	SFCGSPYSW	9	0.0710
95	19	NUC	46	SFLPSDFF	8	
80	16	POL	765	SFVYVPSAL	9	
95	19	POL	413	SWLSLDVSAAF	11	
100	20	ENV	334	SWLSLLVPF	9	0.3900

HBV A24 Motif With Binding Information

Conservancy	Freq.	Protein	Position	Sequence	AA	A*2401
95	19	POL	392	SWPKFAVPNL	10	5.6000
100	20	ENV	197	SWWTSLNF	8	
95	19	ENV	197	SWWTSLNFL	9	0.3800
90	18	POL	537	SYMDDVVL	8	
75	15	POL	4	SYQHFRKL	8	
75	15	POL	4	SYQHFRKLL	9	0.0051
75	15	POL	4	SYQHFRKLLL	10	0.0660
75	15	POL	4	SYQHFRKLLLL	11	
75	15	NUC	138	TFGRETVL	8	
75	15	NUC	138	TFGRETVLEYL	11	
95	19	POL	657	TFSPTYKAF	9	0.0060
95	19	POL	657	TFSPTYKAFL	10	0.0043
95	19	POL	686	VFADATPTGW	10	0.0180
75	15	X	131	VFVLGGCRHKL	11	
90	18	NUC	102	WFHISCLTF	9	0.0300
95	19	ENV	345	WFVGLSPTVW	10	0.0120
95	19	ENV	345	WFVGLSPTVWL	11	
95	19	ENV	237	WMCLRRFI	8	
95	19	ENV	237	WMCLRRFII	9	
95	19	ENV	237	WMCLRRFIIF	10	0.0013
95	19	ENV	237	WMCLRRFIIFL	11	
85	17	ENV	359	WMMWYWGPSL	10	
95	19	ENV	198	WWTSLNFL	8	
				108	3	

Table XIXa

HBV DR-Super Motif

Protein	Core Sequence	Core Freq	Core Conservancy (%)	Exemplary Sequence	Position in HBV Poly-Protein	Exemplary Sequence Frequency	Exemplary Sequence Conservancy (%)
POL	FAAPFTQCG	19	95	LLGFAAPFTQCGYPA	628	19	95
POL	FADATPTGW	19	95	COVFADATPTGWGLA	684	16	80
POL	FAVPNLQSL	19	95	WPKFAVPNLQSLTNL	393	19	95
NUC	FGRETVLEY	15	75	CLTFGRETVLEYLVS	136	14	70
POL	FGVEPSGSG	15	75	RRSFGVEPSGSGHID	252	6	30
NUC	FHISCLTFG	18	90	LLWFHISCLTFGRET	100	17	85
NUC	FHLCLISC	16	80	MQLFHLCLISCSCP	1	10	50
ENV	FILLLCLIF	16	80	IFLFILLLCLIFLLV	245	16	80
ENV	FLFILLLCL	16	80	FIIFLFILLLCLIFL	243	16	80
ENV	FLGPLLVLQ	15	75	TSGFLGPLLVLQAGF	168	15	75
ENV	FLLTRILTI	16	80	AGFFLLTRILTIPQS	180	16	80
ENV	FLLVLLDYQ	19	95	CLIFLLVLLDYQGML	253	19	95
ENV	FPAGGSSG	15	75	GLYFPAGGSSSGTVN	127	11	55
ENV	FPDHQLDPA	18	90	LGFFPDHQLDPAFGA	22	9	45
POL	FPHCLAFSY	19	95	RRAFPHCLAFSYMDD	527	19	95
POL	FRKPMGVG	16	80	ILGFRKPMGVGLSP	498	13	65
POL	FRKLPVNRP	16	80	KQCFRKLPVNRPIDW	616	9	45
X	FSSAGPCAL	19	95	VCAFSSAGPCALRFT	60	18	90
ENV	FSWLSLLVP	20	100	SVRFSWLSLLVPFVQ	330	16	80
POL	FTFSPTYKA	19	95	KQAFTFSPTYKAFLC	653	12	60
POL	FTGLYSSTV	18	90	VGNFTGLYSSTVPVF	56	11	55
POL	FTSAICSVV	19	95	LAQFTSAICSVVRRA	515	19	95
ENV	FVGLSPTVW	19	95	VQWFVGLSPTVWLSV	343	14	70
X	FVLGGCRHK	18	90	LKVFVLGGCRHKLVC	129	14	70
ENV	FVQWFVGLS	19	95	LVPFVQWFVGLSPTV	339	19	95
POL	FVYVPSALN	18	90	GTSFVYVPSALNPAD	763	16	80
POL	IDWKVCQRI	17	85	NRPIDWKVCQRIVGL	614	16	80
ENV	IFLFILLLC	16	80	RFIIFLFILLLCLIF	242	15	75
ENV	IFLLVLLDY	19	95	LCLIFLLVLLDYQGM	252	19	95
POL	IGTDNSVVL	16	80	AKLIGTDNSVVLSRK	731	13	65
POL	IHTAELLAA	17	85	PLPIHTAELLAACFA	711	16	80
ENV	IIFLFILLL	16	80	RRFIIFLFILLLCLI	241	15	75
ENV	ILLLCLIFL	20	100	FLFILLLCLIFLLVL	246	16	80
POL	ILRGTSFVY	16	80	ANWILRGTSFVYVPS	757	16	80
NUC	ILSTLPETT	20	100	NAPILSTLPETTVVR	165	19	95
ENV	IPIPSSWAF	20	100	CTCIPIPSSWAFARF	321	8	40
NUC	IRTPPAYRP	19	95	GVWIRTPPAYRPPNA	123	19	95
POL	LAACFARSR	17	85	AELLAACFARSRSGA	717	16	80
POL	LAFSYMDDV	18	90	PHCLAFSYMDDVVLG	531	18	90
POL	LAQFTSAIC	19	95	PFLLAQFTSAICSVV	512	19	95
NUC	LCLGWLWGM	17	85	ASKLCLGWLWGMDID	19	17	85
ENV	LCLIFLLVL	20	100	ILLLCLIFLLVLLDY	249	19	95
X	LCLRPVGAE	19	95	RDVLCLRPVGAESRG	13	18	90
POL	LCQVFADAT	19	95	RPGLCQVFADATPTG	680	11	55
ENV	LDSWWTSLN	19	95	PQSLDSWWTSLNFLG	192	17	85
NUC	LDTASALYR	17	85	RDLLDTASALYREAL	28	16	80
POL	LDVSAAFYH	19	95	WLSLDVSAAFYHIPL	425	11	55
ENV	LDYQGMLPV	18	90	LVLLDYQGMLPVCPL	258	18	90
POL	LEEELPRLA	18	90	AGPLEEELPRLADEG	18	13	65
ENV	LFILLLCLI	16	80	IIFLFILLLCLIFLL	244	16	80
POL	LGAKSVQHL	17	85	DVVLGAKSVQHLESL	541	16	80
POL	LGFAAPFTQ	19	95	VGLLGFAAPFTQCGY	626	19	95
POL	LGFRKPMG	19	95	PILGFRKPMGVGL	496	13	65
POL	LGNLNVSIP	19	95	DLNLGNLNVSIPWTH	40	19	95

HBV DR-Super Motif:

Protein	Core Sequence	Core Freq.	Core Conservancy (%)	Exemplary Sequence	Position In HBV Poly-Protein	Exemplary Sequence Frequency	Exemplary Sequence Conservancy (%)
ENV	LGPLLVLQA	19	95	SGFLGPLLVLQAGFF	169	15	75
POL	LHPAAMPHL	20	100	HLPLHPAAMPHLLVG	425	9	45
ENV	LIFLLVLLD	19	95	LLCLIFLLVLLDYQG	251	19	95
POL	LKLMPARIF	15	75	KRRLKLMPARFYPN	104	7	35
X	LKVFVLGGC	15	75	EIRLKVFVLGGCRHK	126	13	65
POL	LLAQFTSAI	19	95	SPFLLAQFTSAICSV	511	19	95
NUC	LLDTASALY	17	85	IRDLLDTASALYREA	56	9	45
POL	LLGCAANWI	16	80	FPWLLGCAANWILRG	749	15	75
POL	LLGFAAPFT	19	95	IVGLLGFAAPFTQCG	625	18	90
ENV	LLGWSPQAQ	17	85	HGGLLGWSPQAQGIL	60	15	75
ENV	LLLCLIFLL	20	100	LFILLLCLIFLLVLL	247	16	80
NUC	LLSFLPSDF	19	95	SVELLSFLPSDFFPS	41	11	55
POL	LLSLGIHLN	19	95	TNFLLSLGIHLNPNK	560	15	75
POL	LLSSNLSWL	18	90	LTNLLSSNLSWLSLD	404	18	90
ENV	LLTRILTIP	16	80	GFFLLTRILTIPQSL	181	16	80
ENV	LLVLQAGFF	19	95	LGPLLVLQAGFFLLT	172	18	90
ENV	LLVPFVQWF	20	100	WLSLLVPFVQWFVGL	335	19	95
NUC	LLWFHISCL	18	90	IRQLLWFHISCLTFG	126	13	65
POL	LMPLYACIQ	19	95	YPALMPLYACIQSKQ	640	11	55
POL	LNLGNLNVS	19	95	AEDLNLGNLNVSIPW	38	19	95
POL	LNPNKTKRW	15	75	GIHLNPNKTKRWGYS	567	15	75
POL	LNRRVAEDL	17	85	DEGLNRRVAEDLNLG	30	12	60
POL	LNVSIPWTH	19	95	LGNLNVSIPWTHKVG	43	19	95
NUC	LPETTVVRR	19	95	LSTLPETTVVRRRGR	169	16	80
ENV	LPIFFCLWV	20	100	LPLLPIFFCLWVYIZ	376	13	65
POL	LPIHTAELL	17	85	VAPLPIHTAELLAAC	709	9	45
POL	LPVNRPIDW	16	80	FRKLPVNRPIDWKVC	608	15	75
POL	LQFRNSKPC	18	90	CWWLQFRNSKPCSDY	312	10	50
X	LRGLPVCAF	19	95	HLSLRGLPVCAFSSA	52	18	90
X	LRPVGAESR	18	90	VLCLRPVGAESRGRP	15	18	90
NUC	LRQAILCWG	18	90	HTALRQAILCWGELM	52	18	90
ENV	LRRFIFLF	15	75	WMCLRRFIFLFILL	237	15	75
NUC	LSFLPSDFF	19	95	VELLSFLPSDFFPSI	42	10	50
POL	LSLDVSAAF	19	95	LSWLSLDVSAAFYHI	423	11	55
ENV	LSLLVPFVQ	20	100	FSWLSLLVPFVQWFV	333	19	95
X	LSLRGLPVC	19	95	GAHLSLRGLPVCAFS	50	18	90
POL	LSPFLLAQF	19	95	GVGLSPFLLAQFTSA	507	16	80
POL	LSRKYTSFP	17	85	SVVLSRKYTSFPWLL	739	17	85
POL	LSSNLSWLS	18	90	TNLLSSNLSWLSLDV	405	18	90
ENV	LSVPNPLGF	15	75	GTNLSVPNPLGFFPD	13	14	70
POL	LSWLSLDVS	20	100	SSNLSWLSLDVSAAF	409	17	85
ENV	LTIPQSLDS	18	90	TRILTIPQSLDSWWT	186	15	75
POL	LTNLLSSNL	18	90	LQSLTNLLSSNLSWL	401	18	90
ENV	LTRILTIPQ	16	80	FFLLTRILTIPQSLD	182	15	75
POL	LVDKNPHNT	20	100	GVFLVDKNPHNTTES	372	11	55
NUC	LVSFGVWIR	18	90	LEYLVSFGVWIRTPP	145	14	70
POL	LVVDFSQFS	20	100	ESRLVVDFSQFSRGN	374	9	45
NUC	LWFHISCLT	17	85	RQLLWFHISCLTFGR	98	17	85
NUC	LWGMDIDPY	17	85	LGWLWGMDIDPYKEF	24	17	85
POL	LWKAGILYK	20	100	LHTLWKAGILYKRET	148	18	90
NUC	LYREALESP	17	85	ASALYREALESPEHC	34	17	85
POL	LYSHPIILG	16	80	KLHLYSHPIILGFRK	489	16	80
POL	MDDVVLGAK	18	90	FSYMDDVVLGAKSVQ	536	18	90
POL	MGVGLSPFL	16	80	KIPMGVGLSPFLAQ	503	16	80

HBV DR-Super Motif

Protein	Core Sequence	Core Freq	Core Conservancy (%)	Exemplary Sequence	Position In HBV Poly-Protein	Exemplary Sequence Frequency	Exemplary Sequence Conservancy (%)
POL	MPHLLVGSS	17	85	PAAMPHLLVGSSGLS	430	8	40
ENV	MQWNSTTFH	16	80	PQAMQWNSTTFHQTL	106	8	40
X	MSTTDLEAY	15	75	LSAMSTTDLEAYFKD	100	9	45
ENV	MWYWGPSLY	17	85	IWMMWYWGPSLYNIL	369	9	45
X	VCAFSSAGP	19	95	GLPVCAFSSAGPCAL	57	18	90
POL	VCQRIVGLL	17	85	DWKVCQRIVGLLGFA	618	17	85
POL	VFADATPTG	19	95	LCQVFADATPTGWGL	683	19	95
ENV	VGLSPTVWL	19	95	QWFVGLSPTVWLSVI	344	14	70
POL	VGPLTVNEK	17	85	QQYVGPLTVNEKRRL	93	8	40
POL	VHFASPLHV	16	80	PDRVHFASPLHVAWR	816	12	60
X	VLCLRPVGA	19	95	ARDVLCLRPVGAESR	12	14	70
POL	VLGAKSVQH	19	95	DDVVLGAKSVQHLES	540	16	80
X	VLHKRTLGL	17	85	LPKVLHKRTLGLSAM	89	11	55
POL	VPNLQSLTN	19	95	KFAVPNLQSLTNLLS	395	19	95
NUC	VQASKLCLG	16	80	CPTVQASKLCLGWLW	14	15	75
ENV	VRFSWLSLL	16	80	WASVRFSWLSLLVPF	328	13	65
POL	VRRAFPHCL	19	95	CSVVRRAFPHCLAFS	523	19	95
POL	VSIPWTHKV	20	100	NLNVSIPWTHKVGNF	45	19	95
NUC	VWRTPPAY	19	95	SFGVWRTPPAYRPP	121	18	90
POL	VYVPSALNP	18	90	TSFVYVPSALNPADD	764	16	80
NUC	WFHISCLTF	18	90	QLLWFHISCLTFGRE	99	17	85
ENV	WFVGLSPTV	19	95	FVQWFVGLSPTVWLS	342	19	95
POL	WLRGTSFV	16	80	AANWILRGTSFVYVP	756	14	70
NUC	WRTPPAYR	19	95	FGVWRTPPAYRPPN	122	19	95
POL	WKAGILYKR	20	100	HTLWKAGILYKRETT	149	18	90
POL	WLLGCAANW	16	80	SFPWLLGCAANWILR	748	15	75
POL	WLSLDVSAA	19	95	NLSWLSLDVSAAFYH	411	17	85
ENV	WLSLLVPFV	20	100	RFSWLSLLVPFVQWF	332	20	100
POL	WPKFAVPNL	19	95	RVSWPKFAVPNLQSL	390	11	55
POL	YMDDVVLGA	18	90	AFSYMDDVVLGAKSV	535	18	90
POL	YPALMPLYA	19	95	QCGYPALMPLYACIQ	637	19	95
ENV	YQGMLPVCP	18	90	LLDYQGMLPVCPLIP	260	10	50
NUC	YRPPNAPIL	20	100	PPAYRPPNAPILSTL	129	19	95
ENV	YRWMCLRRF	19	95	CPGYRWMCLRRFIIF	232	19	95
POL	YSHPIILGF	16	80	LHLYSHPIILGFRKI	490	16	80
POL	YSLNFMGYV	15	75	RWGYSLNFMGYVIGS	588	11	55
POL	YVPSALNPA	18	90	SFVYVPSALNPADDP	765	16	80
ENV	FFCLWVYIZ	20	100		382		
ENV	MGTNLSVPN	15	75		12		

Table XIXb

HBV DR-Super Motif With Binding Data

Core Sequence	Exemplary Sequence	DR1	DR2w2β1	DR2w2β2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	DR6w19	DR7	DR8w2	DR9	DRw53
FAAPFTQCG	LLGFAAPFTQCGYPA													
FADATPTGW	CQVFADATPTGWGLA						0 2800							
FAVPNLQSL	WPKFAVPNLQSLTNL	0 0007		0 0013		0 0023		0 0002			0.0008			0 0180
FGRETVLEY	CLTFGRETVLEYLVS													
FGVEPSGSG	FRSFGVEPSGSGHD													
FHSCLTFG	LLWFHSCLTFGRET													
FHLCLIISC	MQLFHLCLIISCSCP													
FILLLCLIF	IFLFILLLCLIFLLV	0 0005				0 0041					0 0018			
FLFILLLCL	FIIFLFILLLCLIFL													
FLGPLLVLQ	TSGFLGPLLVLQAGF													
FLLTRILTI	AGFFLLTRILTIPQS	4 6000	0 0420	0 0190	0 0040	5 3000	0.1500	3 6000	0 0700	0 3700	3 1000	0 2600	1 3000	
FLLVLLDYQ	CLIFLLVLLDYQGML													
FPAGGSSSG	GLYFPAGGSSSGTVN													
FPDHQLDPA	LGFFPDHQLDPAFGA													
FPHCLAFSY	RRAFPHCLAFSYMDD	0 0010		0 0010		-0 0009		0 0010			0 0017			
FRKPMGVG	ILGFRKPMGVGLSP													
FRKLPVNRP	KQCFRKLPVNRPIDW	1.5000	0 0022	0 0210	-0 0006	1 2000	0 8500	0 0130	0 0013	0 0043	0 4000	0 0580	0 0250	
FSSAGPCAL	VQAFSSAGPCALRFT	0 2100		0 2600		0 0023		0 0003			0 0200			0 0150
FSWLSLLVP	SVRFSWLSLLVPFVQ	0 9000				0 0099					0 0037			
FTFSPTYKA	KQAFTFSPTYKAFLC	0 5300	0 2400	0 1400	0 0090	1 1000	0 2200	0 2400	0 0024	0 0200	0 3300	0.1200	0 5400	
FTGLYSSTV	VGNFTGLYSSTVPVF	1 7000	0 0100	0 0016		0 0140	0 1700	0 0035		0 0580	0 5600	0 0044	0 3100	
FTSAICSVV	LAQFTSAICSVVRRA	0 0120	0.0065	0 1500	-0 0009	0 0150	0 2800	0 0076	0 0091	0 0010	0 0280	0.0150	0 0880	0 0190
FVGLSPTVW	VQWFVGLSPTVWLSV													
FVLGGCRHK	LKVFVLGGCRHKLVC													
FVQWFVGLS	LVPFVQWFVGLSPTV	0 0130	0 6900	0 0140	-0 0013	0 1500	1 4000	0 3800	0 6600	0 0018	0 0092	0 6600	2.5000	2.6000
FVYVPSALN	GTSFVYVPSALNPAD	0.3500	0 0140	0 0500	-0 0006	0 3800	0 4100	0 0470	-0 0001	0 0001	0 2700	0.0610	0.3400	
IDWKVCQRI	NRPIDWKVCQRIVGL													
IFLFILLLC	RFIIFLFILLLCLIF													
IFLLVLLDY	LCLIFLLVLLDYQGM	0.0016		0 0060		0 0230		0 0017			0.0044			
IGTDNSVVL	AKLIGTDNSVVLSRK													
IHTAELLAA	PLPIHTAELLAACFA	0.0046				0 0490					-0 0003			
IIFLFILLL	RRFIIFLFILLLCLI													
ILLLCLIFL	FLFILLLCLIFLLVL													
IURGTSFVY	ANWILRGTSFVYVPS													
ILSTLPETT	NAPILSTLPETTVVR	0 0009		0 0009		-0 0007		-0 0002			0 0005			0 1600
IPIPSSWAF	CTCIPIPSSWAFARF													
IRTPPAYRP	GVWIRTPPAYRPPNA	0 3700	0 0420	7 2000	0 0120	3 4000	0 5700	0 4800	0 0140	-0 0004	0 2200	0.5300	0 0450	
LAACFARSR	AELLAACFARSRSGA													
LAFSYMDDV	PHCLAFSYMDDVVLG													
LAQFTSAIC	PFLLAQFTSAICSVV	0 1800	0 0270	0 0042	-0 0013	0 0800	0 1200	0 0120	0 0016	0 0800	0 0770	0 0580	0 0590	
LCLGWLWGM	ASKLCLGWLWGMDID	0 0002		-0 0005		0 0017		-0 0002			0 0013			0 0010
LCLIFLLVL	ILLLCLIFLLVLLDY	0 0026		0 0069		0 0320		0 0018			0 0047			
LCLRPVGAE	RDVLCLRPVGAESRG													
LCQVFADAT	RPGLCQVFADATPTG													
LDSWWTSLN	POSLDSWWTSLNFLG													
LDTASALYR	RDLLDTASALYREAL	0 0001				0 0092					0 0770			
LDVSAAFYH	WLSLDVSAAFYHIPL													
LDYQGMLPV	LVLLDYQGMLPVCPL	0.0034				-0 0013					0.0011			
LEEELPRLA	AGPLEEELPRLADEG				0 0022									
LFILLLCLI	IIFLFILLLCLIFLL													
LGAKSVQHL	DVVLGAKSVQHLESL													
LGFAAPFTQ	VGLLGFAAPFTQCGY	0.0470	0.3100	0.0008		-0.0014		-0.0004		-0.0001	0.0014		0.5700	
LGFRKPMG	PILGFRKPMGVGL													
LGNLNVSIP	DLNLGNLNVSIPWTH	0.0038				0.0240					0.0010			

HBV DR-Super Motif With Binding Data

Core Sequence	Exemplary Sequence	DR1	DR2w201	DR2w202	DR3	DR4w4	DR4w15	DR5w11	DR5w12	DR6w19	DR7	DR8w2	DR9	DRw53
LGPLLVLQA	SGFLGPLLVLQAGFF													
LHPAAMPHL	HLPLHPAAMPHLLVG													
LIFLLVLLD	LLCLIFLLVLLDYQG													
LKUMPARF	KRRLKUMPARFYPN													
LKVFVLGGC	EIRLKVFVLGGCRHK													
LLAQFTSAI	SPFLLAQFTSAICSV	0.1200	0 0200	0 0085	-0 0013	0 0740	0 1900	-0 0002	-0 0013	0 0540	0 0330	0.0014	0 0380	0 2000
LLDTASALY	IRDLLDTASALYREA													
LLGCAANWI	FPWLLGCAANWILRG													
LLGFAAPFT	MGLLGFAAPFTQCG	0 0200		-0 0005		-0 0007		-0.0002			0 0009			0 0067
LLGWSPQAQ	HGGLLGWSPQAQGIL													
LLLCLIFLL	LFILLLCLIFLLVLL													
LLSFLPSDF	SVELLSFLPSDFFPS													
LLSLGIHLN	TNFLLSLGIHLNPNK	3 5000	0 0410	0 1200		0 0220	0 3600	0 0053		0 0160	0 2200	0 0032	0 3800	
LLSSNLSWL	LTNLLSSNLSWLSLD	0 0010		0 0083		0 0160		0 0013			0 0019			0 0200
LLTRILTIP	GFFLLTRILTIPQSL	0 4300	0 0150	0 0110		3 1000	0 4500	2 3000		0 0780	3 5000	1.6000	0 5500	
LLVLQAGFF	LGPLLVLQAGFFLLT													
LLVPFVQWF	WLSLLVPFVQWFVGL													
LLWFHISCL	IRQLLWFHISCLTFG													
LMPLYACIQ	YPALMPLYACIQSKQ	0 2400				0 0014					0 0011			
LNLGNLNVS	AEDLNLGNLNVSIPW	0 0001		-0 0005		-0 0007		-0 0002			-0 0003			0 0170
LNPNKTKRW	GIHLNPNKTKRWGYS													
LNRRVAEDL	DEGLNRRVAEDLNLG													
LNVSIPWTH	LGNLNVSIPWTHKVG													
LPETTVRR	LSTLPETTVRRRGR													
LPIFFCLWV	LPLLPIFFCLWVYIZ													
LPIHTAELL	VAPLPIHTAELLAAC													
LPVNRPIDW	FRKLPVNRPIDWKVC													
LQFRNSKPC	CWWLQFRNSKPCSDY													
LRGLPVCAF	HLSLRGLPVCAFSSA	1 3000				0 0028					0 0130			
LRPVGAESR	VLCLRPVGAESRGRP													
LRQAILCWG	HTAURQAILCWGELM													
LRRFIFLF	WMCLRRFIIFLFILL													
LSFLPSDFF	VELLSFLPSDFFPSI													
LSLDVSAAF	LSWLSLDVSAAFYHI													
LSLLVPFVQ	FSWLSLLVPFVQWFV													
LSLRGLPVC	GAHLSLRGLPVCAFS	0 7800		0 0042	-0 0041	0 0011		0 0025			0 0077			0 0150
LSPFLLAQF	GVGLSPFLLAQFTSA													
LSRKYTSFP	SVVLSRKYTSFPWLL	0 0005		0 0057	0 2100	-0 0016		0 5300			0 0130			
LSSNLSWLS	TNLLSSNLSWLSLDV	0 0016		-0 0005		0 1300		0 0006			0 0019			0 0410
LSVPNPLGF	GTNLSVPNPLGFFPD													
LSWLSLDVS	SSNLSWLSLDVSAAF	0.1400	0 0030	-0.0005	1 5000	0 2700		0 0046	0 0180	0 1000	0 0039	0 0460	0 0110	6 2000
LTIPQSLDS	TRILTIPQSLDSWWT													
LTNLLSSNL	LQSLTNLLSSNLSWL	2 5000	0 4400	0 0200	-0 0013	4 8000	0 8100	0 0680	0 7500	0 0260	0 1500	0 0880	0.1100	
LTRILTIPQ	FFLLTRILTIPQSLD													
LVDKNPHNT	GVFLVDKNPHNTTES													
LVSFGVWIR	LEYLVSFGVWIRTPP													
LVVDFSQFS	ESRLVVDFSQFSRGN	0 0007	0 0074	-0 0010	2 6000			-0 0004		0 0040	-0 0014	0 0029		
LWFHISCLT	ROLLWFHISCLTFGR	0 0002		0 0009		0 0140		0 0011			0 0061			0 0096
LWGMDIDPY	LGWLWGMDIDPYKEF	0 0004		0.0006	0 0200	0 0280		-0 0002			0 0004			0 0430
LWKAGILYK	LHTLWKAGILYKRET													
LYREALESP	ASALYREALESPEHC													
LYSHPIILG	KLHLYSHPIILGFRK													
MDDVVLGAK	FSYMDDVVLGAKSVQ													
MGVGLSPFL	KIPMGVGLSPFLLAQ													

HBV DR-Super Motif With Binding Data

Core Sequence	Exemplary Sequence	DR1	DR2w2β1	DR2w2β2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	DR6w19	DR7	DR8w2	DR9	DRw53
MPHLLVGSS	PAAMPHLLVGSSGLS													
MQWNSTTFH	PQAMQWNSTTFHQTL	0 0012				0 0300					0.1200			
MSTTDLEAY	LSAMSTTDLEAYFKD													
MWYWGPSLY	IWMMWYWGPSLYNIL													
VCAFSSAGP	GLPVCAFSSAGPCAL													
VCQRIVGLL	DWKVCQRIVGLLGFA	0 0120		-0 0026		0 0030		0 2500			0 0018			0 0130
VFADATPTG	LCQVFADATPTGWGL	0 0020				0 9600					0 0013			
VGLSPTVWL	QWFVGLSPTVWLSVI													
VGPLTVNEK	QQYVGPLTVNEKRRL													
VHFASPLHV	PDRVHFASPLHVAWR	0 0510	0 0290	0 0008		0 0008	0 5400	0.0008		0 0190	0 0810	0 0035	0 2400	
VLCLRPVGA	ARDVLCLRPVGAESR													
VLGAKSVQH	DDVVLGAKSVQHLES													
VLHKRTLGL	LPKVLHKRTLGLSAM													
VPNLQSLTN	KFAVPNLQSLTNLLS	0 0180	0 0005	-0 0003		0 1300		0 0043		0 0088	-0 0003		0 0056	
VQASKLCLG	CPTVQASKLCLGWLW													
VRFSWLSLL	WASVRFSWLSLLVPF													
VRRAFPHCL	CSVVRRAFPHCLAFS	0 1000	0 1024	0 0770	0 0032	0 0016	-0 2200	0 0008	-0 0013	0 0540	0 0590	0 0250	1 2000	0 0460
VSIPWTHKV	NLNVSIPWTHKVGNF	0 0001		-0 0005	-0 0041	-0 0007		-0 0002			0 0005			0 0009
VWIRTPPAY	SFGVWIRTPPAYRPP	0 0094	0 0110	0.4300	-0 0009	0 0780	0 6300	0 0260	0 0071	0 0002	0 0240	0 2500	0 0800	0 0016
VYVPSALNP	TSFVYVPSALNPADD													
WFHISCLTF	QLLWFHISCLTFGRE													
WFVGLSPTV	FVQWFVGLSPTVWLS	0 4700	0 0035	0 0160	-0 0013	0 0130		0 0072	0 0021	0 0190	0 0690	0 0180	0 0410	0 0044
WILRGTSFV	AANWILRGTSFVYVP	0 0920	0 0240	0 0061	0 0023	0 0510	0 2500	0 0140	0 3700	0 0250	0 5800	0 2500	0 2700	
WIRTPPAYR	FGVWIRTPPAYRPPN													
WKAGILYKR	HTLWKAGILYKRETT													
WLLGCAANW	SFPWLLGCAANWILR													
WLSLDVSAA	NLSWLSLDVSAAFYH	0 1400	0 0003	-0 0005	1 3000	0 2900		0 0033	0 0022	0 0330	0 0041	0 0150	0 0620	2 4000
WLSLLVPFV	RFSWLSLLVPFVQWF	0 0430		0 0009		-0 0007		0 0002			0 0005			0 0031
WPKFAVPNL	RVSWPKFAVPNLQSL													
YMDDVVLGA	AFSYMDDVVLGAKSV	0 0027		-0 0005	0 0130	2 9000		0 0006			-0 0003			-0 0005
YPALMPLYA	QCGYPALMPLYACIQ	0 0062		0 0018		0 0068		0 0023			0 0006			
YQGMLPVCP	LLDYQGMLPVCPLIP													
YRPPNAPIL	PPAYRPPNAPILSTL	0 0056		-0 0005		0 0038		0 0022			0 0024			0 0015
YRWMCLRRF	CPGYRWMCLRRFIF													
YSHPIILGF	LHLYSHPIILGFRKI	0.0220	0 0340	0 0400	0 0040	0 6800	0 1600	0 0410	0 0310	0 0002	0 0006	0.0610	0.0490	
YSLNFMGYV	RWGYSLNFMGYVIGS													
YVPSALNPA	SFVYVPSALNPADDP													
FFCLWVYIZ														
MGTNLSVPN														
148														

Table XXa

HBV DR-3A Motif

Protein	Core Sequence	Core Freq	Core Conservancy (%)	Exemplary Sequence	Position In Poly-Protein	Exemplary Sequence Frequency	Exemplary Sequence Conservancy (%)
ENV	FFPDHQLDP	19	95	PLGFFPDHQLDPAFG	10	9	95
NUC	FGRETVLEY	15	75	CLTFGRETVLEYLVS	136	14	75
POL	FGVEPSGSG	15	75	RRSFGVEPSGSGHID	241	6	75
POL	FLVDKNPHN	20	100	GGVFLVDKNPHNTTE	360	11	100
POL	IGTDNSVVL	16	80	AKLIGTDNSVVLSRK	731	13	80
POL	LEEELPRLA	18	90	AGPLEEELPRLADEG	18	13	90
POL	LPLDKGIKP	20	100	TKYLPLDKGIKPYYP	120	20	100
POL	LSLDVSAAF	19	95	LSWLSLDVSAAFYHI	412	11	95
POL	LVVDFSQFS	20	100	ESRLVVDFSQFSRGN	374	9	100
NUC	LYREALESP	17	85	ASALYREALESPEHC	34	17	85
NUC	MDIDPYKEF	17	85	LWGMDIDPYKEFGAS	27	9	85
POL	VAEDLNLGN	20	100	NRRVAEDLNLGNLNV	34	17	100
POL	VFADATPTG	19	95	LCQVFADATPTGWGL	683	19	95
ENV	VLLDYQGML	19	95	FLLVLLDYQGMLPVC	256	18	95
POL	YMDDVVLGA	18	90	AFSYMDDVVLGAKSV	535	16	90
		15					

TABLE XXb

V DR 3A Motif

Core Sequence	Exemplary Sequence	DR1	DR2w2β1	DR2w2β2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	DR6w1	DR7	DR8W2	DR9	DRW53
FFPDHQLDP	PLGFFPDHQLDPAFG													
FGRETVLEY	CLTFGRETVLEYLVS													
FGVEPSGSG	RRSFGVEPSGSGHD													
FLVDKNPHN	GGVFLVDKNPHNTTE				0 0790									
IGTDNSVVL	AKLIGTDNSVVLSRK													
LEEELPRLA	AGPLEEELPRLADEG				0 0022									
LPLDKGIKP	TKYLPLDKGIKPYYP				-0 0017									
LSLDVSAAF	LSWLSLDVSAAFYHI													
LVVDFSQFS	ESRLVVDFSQFSRGN	0 0007	0 0074	-0 0010	2 6000			-0 0004		0 4000	-0 0014	0 0029		
LYREALESP	ASALYREALESPEHC													
MDIDPYKEF	LWGMDIDPYKEFGAS													
VAEDLNLGN	NRRVAEDLNLGNLNV				0 1400									
VFADATPTG	LCQVFADATPTGWGL	0.0020				0 9600					0 0013			
VLLDYQGML	FLLVLLDYQGMLPVC				0 0170									
YMDDVVLGA	AFSYMDDVVLGAKSV	0 0027		-0 0005	0 0130	2 9000		0 0006			-0 0003			-0 0005
15														

Table XXc

HBV DR-3B Motif

Protein	Core Sequence	Core Freq	Core Conservancy (%)	Exemplary Sequence	Position In HBV Poly-Protein	Exemplary Sequence Frequency	Exemplary Sequence
X	AHLSLRGLP	18	90	DIIGAHLSLRGLPVCA	48	18	90 00
POL	FSPTYKAFL	19	95	AFTFSPTYKAFLCKQ	655	11	55 00
POL	IPWTHKVGN	20	100	NVSIPWTHKVGNFTG	47	20	100 00
POL	LTVNEKRRL	17	85	VGPLTVNEKRRLKLI	96	12	60 00
X	VGAESRGRP	19	95	LRPVGAESRGRPVSG	18	7	35 00
POL	VVLSRKYTS	18	90	DNSVVLSRKYTSFPW	737	17	85 00

Table XXd

HBV DR-3B Motif With Binding Information

Core Sequence	Exemplary Sequence	DR1	DR2w2β1	DR2w2β2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	DR6w19	DR7	DR8w2	DR9	DRw53
AHLSLRGLP	DHGAHLSLRGLPVCA				0 0035									
FSPTYKAFL	AFTFSPTYKAFLCKQ													
IPWTHKVGN	NVSIPWTHKVGNFTG													
LTVNEKRRL	VGPLTVNEKRRLKLI	0 0006	0 0022	0 0047	2 2000			0 0030		0 0009	-0 0014	0 0092		
VGAESRGRP	LRPVGAESRGRPVSG				-0 0017									
VVLSRKYTS	DNSVVLSRKYTSFPW													

TABLE XXI. Population coverage with combined HLA Supertypes

HLA-SUPERTYPES	PHENOTYPIC FREQUENCY					
	Caucasian	North American Black	Japanese	Chinese	Hispanic	Average
a. Individual Supertypes						
A2	45.8	39.0	42.4	45.9	43.0	43.2
A3	37.5	42.1	45.8	52.7	43.1	44.2
B7	38.6	52.7	48.8	35.5	47.1	44.7
A1	47.1	16.1	21.8	14.7	26.3	25.2
A24	23.9	38.9	58.6	40.1	38.3	40.0
B44	43.0	21.2	42.9	39.1	39.0	37.0
B27	28.4	26.1	13.3	13.9	35.3	23.4
B62	12.6	4.8	36.5	25.4	11.1	18.1
B58	10.0	25.1	1.6	9.0	5.9	10.3
b. Combined Supertypes						
A2, A3, B7	83.0	86.1	87.5	88.4	86.3	86.2
A2, A3, B7, A24, B44, A1	99.5	98.1	100.0	99.5	99.4	99.3
A2, A3, B7, A24, B44, A1, B27, B62, B58	99.9	99.6	100.0	99.8	99.9	99.8

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Table XXII HBV ANALOGS

AA	Sequence	Fixed Nomen.	A1 Motif	A2 Super Motif	A3 Super Motif	A24 Motif	B7 Super Motif	1° Anchor Fixer	Analog
10	CILLLCLIFL		N	Y	N	N	N	No	A
9	RMTGGVFLV	VM2.V9	N	Y	N	N	N	1	A
9	LMPFVQWFV	VM2.V9	N	Y	N	N	N	1	A
9	RLTGGVFLV	VL2.V9	N	Y	N	N	N	1	A
9	GLCQVFADV	L2.AV9	N	Y	N	N	N	1	A
9	WLLRGTSFV	IL2.V9	N	Y	N	N	N	1	A
9	NLGNLNVSV	L2.IV9	N	Y	N	N	N	1	A
9	YLPSALNPV	VL2.AV9	N	Y	N	N	N	1	A
9	GLWIRTPPV	VL2.AV9	N	Y	N	N	N	1	A
9	RLSWPKFAV	VL2.V9	N	Y	N	N	N	1	A
9	ILGLLGFAV	VL2.AV9	N	Y	N	N	N	1	A
9	RMLTIPQSV	IM2.LV9	N	Y	N	N	N	1	A
9	SLDSWWTSV	L2.LV9	N	Y	N	Y	N	1	A
10	FMLLLCLIFL	IM2.L10	N	Y	N	N	N	1	A
10	LMLQAGFFLV	VM2.LV10	N	Y	N	N	N	1	A
10	SMLSPFLPLV	IM2.LV10	N	Y	N	N	N	1	A
10	LMLLDYQGMV	VM2.LV10	N	Y	N	N	N	1	A
10	FLGLSPTVWV	VL2.LV10	N	N	N	N	Y		A
8	FPAAMPHL		N	N	N	N	Y		A
8	HPFAMPHL		N	N	N	N	Y		A
8	HPAAMPHI		N	N	Y	N	N		A
8	FMFSPTYK		N	N	Y	N	N		A
8	FVFSPTYK		N	Y	N	N	N	1	A
9	FLLTRILTV	L2.IV9	N	Y	N	N	N	1	A
9	ALMPLYACV	L2.IV9	N	Y	N	N	N	1	A
9	LLAQFTSAV	L2.IV9	N	Y	N	N	N	1	A
9	LLPFVQWFV	VL2.V9	N	Y	N	N	N	1	A
9	FLLAQFTSV	L2.AV9	N	Y	N	N	N	1	A
9	KLHLYSHPV	L2.IV9	N	Y	N	N	N	No	A
9	KLFLYSHPI		N	Y	N	N	N	1	A
9	LLSSNLSWV	L2.LV9	N	Y	N	N	N	1	A
9	FLLSLGIHV	L2.LV9	N	Y	N	N	N	1	A
9	MMWYWGPSV	M2.LV9	N	Y	N	N	N	1	A
9	VLQAGFFLV	L2.LV9	N	Y	N	N	N	1	A
9	PLLPIFFCV	L2.LV9	N	Y	N	N	N	No	A
9	FLLPIFFCL		N	Y	N	N	N	1	A
9	VLLDYQGMV	L2.LV9	N	Y	N	N	N	No	A
9	YMFDVVLGA		N	Y	N	N	N	1	A
9	GLLGWSPQV	L2.AV9	N	N	N	N	Y		A
9	FPAAMPHLL		N	N	N	N	Y		A
9	HPFAMPHLL		N	N	N	N	Y		A
9	HPAAMPHLI		N	N	N	N	Y		A
9	FPVCAFSSA		N	N	N	N	Y		A
9	LPFCAFSSA		N	N	N	N			
9	LPVCAFSSI								

HBV ANALOGS

AA	Sequence	Fixed Nomen.	A1 Motif	A2 Super Motif	A3 Super Motif	A24 Motif	B7 Super Motif	1° Anchor Fixer	Analog
9	FPALMPLYA		N	N	N	N	Y		A
9	YPFLMPLYA		N	N	N	N	Y		A
9	YPALMPLYI		N	N	N	N	Y		A
9	FPSRGRLGL		N	N	N	N	Y		A
9	DPFRGRLGL		N	N	N	N	Y		A
9	DPSRGRLGI		N	N	Y	N	N		A
9	SMICSVVRR		N	N	Y	N	N		A
9	SVICSVVRR		N	N	Y	N	N		A
9	KVGNFTGLK		N	N	Y	N	N		A
9	KVGNFTGLR		N	N	Y	N	N		A
9	VVFFSQFSR		N	N	Y	N	N		A
9	SVNRPIDWK		N	N	Y	N	N		A
9	TLWKAGILK		N	N	Y	N	N		A
9	TLWKAGILR		Y	N	Y	N	N		A
9	TMWKAGILY		N	N	Y	N	N		A
9	TVWKAGILY		N	N	Y	N	N		A
9	RMYLHTLWK		N	N	Y	N	N		A
9	RVYLHTLWK		N	N	Y	N	N		A
9	AMTFSPTYK		N	N	Y	N	N		A
9	SVVRRAFPR		N	N	Y	N	N		A
9	SVVRRAFPK		N	N	Y	N	N		A
9	SAIXSVVRR		N	N	N	N	Y	1	A
9	LPVXAFSSA	L2.IV10	N	Y	N	N	N	No	A
10	FLLAQFTSAV		N	Y	N	N	N	No	A
10	YLFTLWKAGI		N	Y	N	N	N	No	A
10	YLLTLWKAGI		N	Y	N	N	N	No	A
10	LLFYQGMLPV		N	Y	N	N	N	1	A
10	LLLYQGMLPV	L2.LV10	N	Y	N	N	N	1	A
10	LLVLQAGFFV	L2.LV10	N	Y	N	N	Y		A
10	ILLLCLIFLV		N	N	N	N	Y		A
10	FPFCLAFSYM		N	N	N	N	Y		A
10	FPHCLAFSYI		N	N	N	N	Y		A
10	FPARVTGGVF		N	N	N	N	Y		A
10	TPFRVTGGVF		N	N	N	N	Y		A
10	TPARVTGGVI		N	N	N	N	Y		A
10	FPCALRFTSA		N	N	N	N	Y		A
10	GPFALRFTSA		N	N	N	N	Y		A
10	GPCALRFTSI		N	N	N	N	Y		A
10	FPAAMPHLLV		N	N	N	N	Y		A
10	HPFAMPHLLV		N	N	N	N	Y		A
10	HPAAMPHLLI		N	N	Y	N	N		A
10	QMFTFSPTYK		N	N	Y	N	N		A
10	QVFTFSPTYK		N	N	Y	N	N		A
10	TMWKAGILYK								
10	TVWKAGILYK								

HBV ANALOGS

AA	Sequence	Fixed Nomen.	A1 Motif	A2 Super Motif	A3 Super Motif	A24 Motif	B7 Super Motif	1° Anchor Fixer	Analog
10	VMGGVFLVDK		N	N	Y	N	N		A
10	VVGGVFLVDK		N	N	Y	N	N		A
10	SMLPETTVVR		N	N	Y	N	N		A
10	SVLPETTVVR		N	N	Y	N	N		A
10	TMPETTVVRR		N	N	Y	N	N		A
10	TVPETTVVRR		N	N	Y	N	N		A
10	HTLWKAGILK		N	N	Y	N	N		A
10	HTLWKAGILR		Y	N	Y	N	N		A
10	HMLWKAGILY		N	N	Y	N	N		A
10	HVLWKAGILY		N	N	Y	N	N		A
10	GMDNSVVLSR		N	N	Y	N	N		A
10	GVDNSVVLSR		N	N	Y	N	N		A
10	GTFNSVVLSR		N	N	Y	N	N		A
10	YMFDVVLGAK		N	N	Y	N	N		A
10	MMWYWGPSLK		N	N	Y	N	N		A
10	MMWYWGPSLR		N	Y	N	N	N		A
9	ILLLXLIFL		N	Y	N	N	N		A
9	LLLXLIFLL		N	Y	N	N	N		A
9	LLXLIFLLV		N	Y	N	N	N		A
9	PLLPIFFXL		N	Y	N	N	N		A
9	ALMPLYAXI		N	Y	N	N	N		A
9	GLXQVFADA		N	N	Y	N	N		A
9	HISXLTFGR		N	N	Y	N	N		A
9	FVLGGXRHK		N	Y	N	N	N		A
10	FILLLXLIFL		N	Y	N	N	N		A
10	ILLLXLIFLL		N	Y	N	N	N		A
10	LLLXLIFLLV		N	Y	N	N	N		A
10	LLPIFFXLWV		N	Y	N	N	N		A
10	QLLWFHISXL		N	Y	N	N	N		A
10	LLGXAANWIL		N	N	Y	N	N		A
10	TSAIXSVVRR		N	N	N	Y	N		A
10	GYRWMXLRRF		N	N	N	N	Y		A
10	GPXALRFTSA		N	N	N	N	Y		A
10	FPHXLAFSYM		N	N	Y	N	N		A
11	HMLWKAGILYK		N	N	Y	N	N		A
11	HVLWKAGILYK		N	N	Y	N	N		A
11	SMLPETTVVRR		N	N	Y	N	N		A
11	SVLPETTVVRR		N	N	Y	N	N		A
11	GMDNSVVLSRK		N	N	Y	N	N		A
11	GVDNSVVLSRK		N	N	Y	N	N		A
11	GTFNSVVLSRK		N	N	N	N	N		A
8	MPLSYQHI		N	N	N	N	Y		A
8	LPIFFCLI		N	N	N	N	Y		A
8	SPFLLAQI		N	N	N	N	Y		A
8	YPALMPLI								

HBV ANALOGS

AA	Sequence	Fixed Nomen.	A1 Motif	A2 Super Motif	A3 Super Motif	A24 Motif	B7 Super Motif	1° Anchor Fixer	Analog
8	VPSALNPI		N	N	N	N	Y		A
9	LPIFFCLWI		N	N	N	N	Y		A
9	LPIHTAELI		N	N	N	N	Y		A
10	VPFVQWFVGI		N	N	N	N	Y		A
11	NPLGFFPDHQI		N	N	N	N	Y		A
11	LPIHTAELLAI		N	Y	N	N	N	Rev3	A
9	FLPSYFPSA	L2.FY5.VA9	N	Y	N	N	N	1	A
10	YLHTLWKAGV	L2.IV10	N	N	Y	N	N		A
11	STLPETYVVRR		N	Y	N	N	N	1	A
9	YMDDVVLGV	M2.AV9	N	N	N	N	Y		A
9	FPIPSSWAF		N	N	N	N	Y		A
9	IPITSSWAF		N	N	N	N	Y		A
9	IPILSSWAF		N	N	N	N	Y		A
9	FPVCLAFSY		N	N	N	N	Y		A
9	FPHCLAFAY		N	N	N	N	Y		A
9	FPIICLAFSL		N	N	N	N	Y		A
9	IPIPMSWAF		N	N	N	N	Y		A
9	FPHCLAFAL		N	Y	N	N	N	No	A
10	FLPSZFFPSV		N	Y	N	N	N	No	A
10	FLPSZFFPSV		N	N	N	N	Y		A
9	IPFPSSWAF		N	N	N	N	Y		A
9	IPIPSSWAI		N	N	N	N	Y		A
9	FPFCLAFSY		N	N	N	N	Y		A
9	FPHCLAFSI		N	N	N	N	Y		A
9	FPHCLAFSA		N	Y	N	N	N	Rev	A
10	FQPSDYFPSV		N	Y	N	N	N		A
9	YLLTRILTI		N	Y	N	N	N		A
9	FLYTRILTI		N	Y	N	N	N		A
9	FLLTYILTI		N	Y	N	N	N		A
9	FLLTRILYI		N	N	Y	N	N		A
11	FLPSDFFPSVR		N	N	N	N	N		A
9	FLPSDFFPS		N	N	N	N	N		A
8	FLPSDFFP		N	Y	N	N	N	Rev	A
10	FLPSDFFPSI	L2.VI10	N	Y	N	N	N	No	A
10	FLPSDYFPSV		N	[redacted]	N	N	N		A
12	YSFLPSDFFPSV		N	N	N	N	N		A
10	YNMGLKFRQL		N	Y	N	Y	N	No	A
9	NMGLKYRQL		N	N	N	N	N		A
10	FLPS(X)YFPSV		N	N	N	N	N		A
10	FLPSD(X)FPSV		N	N	Y	N	N		A
11	FLPSDLLPSVR		N	N	N	N	N		A
12	FLPSDFFPSVRD		N	N	N	N	N		A
12	LSFLPSDFFPSV		N	N	N	N	N		A
11	SFLPSDFFPSV		N	N	N	N	N		A
8	PSDFFPSV	[redacted]							

HBV ANALOGS

AA	Sequence	Fixed Nomen.	A1 Motif	A2 Super Motif	A3 Super Motif	A24 Motif	B7 Super Motif	1° Anchor Fixer	Analog
9	FLMSYFPSV	L2.FY5.V9	N	Y	N	N	N	No	A
9	FLPSYFPSV		N	Y	N	N	N	3	A
10	FLMSDYFPSV		N	Y	N	N	N	No	A
11	CILLLCLIFLL		N	Y	N	N	N	No	A
10	FLPNDFFPSA	L2.SN4.VA10	N	Y	N	N	N	Rev	A
10	FLPDDFFPSA	L2.SD4.VA10	N	Y	N	N	N	Rev	A
10	FLPNDFFPSV		N	Y	N	N	N	No	A
10	FLPSDFFPSA	L2.VA10	N	Y	N	N	N	Rev	A
10	FLPDDFFPSV		N	Y	N	N	N	No	A
10	FLPADFFPSV		N	Y	N	N	N	No	A
10	FLPVDFFPSV		N	Y	N	N	N	No	A
10	FLPADFFPSI	L2.SA4.VI10	N	Y	N	N	N	Rev	A
10	FLPVDFFPSI	L2.SV4.VI10	N	Y	N	N	N	Rev	A
10	FLPSDAFPSV		N	Y	N	N	N	No	A
10	FLPSAFFPSV		N	Y	N	N	N	No	A
10	FLPSDFAPSV		N	Y	N	N	N	No	A
10	FLPSDFFASV		N	Y	N	N	N	No	A
10	FLPSDFFPAV		N	Y	N	N	N	No	A
10	FLASDFFPSV	LA2.V10	N	Y	N	N	N	Rev	A
10	FAPSDFFPSV		N	Y	N	N	N	No	A
10	ALPSDFFPSV		N	Y	N	N	N	No	A
10	YLPSDFFPSV	LM2.V10	N	Y	N	N	N	1	A
10	FMPSDFFPSV		N	Y	N	N	N	No	A
10	FLKSDFFPSV		N	Y	N	N	N	No	A
10	FLPSEFFPSV		N	Y	N	N	N	No	A
10	FLPSDFYPSV		N	Y	N	N	N	No	A
10	FLPSDFFKSV		N	Y	N	N	N	No	A
10	FLPSDFFPKV		N	N	N	N	N		Amidated
10	FLPSDFFPSV(CONH2)		N	N	N	N	N		Amidated
10	VLEYLVSFGV(NH2)		N	N	N	N	N		Amidated
17	ATVELLSFLPSDFFPSV-NH2		N	N	N	N	N		Amidated
16	TVELLSFLPSDFFPSV-NH2		N	N	N	N	N		Amidated
15	VELLSFLPSDFFPSV-NH2		N	N	N	N	N		Amidated
14	ELLSFLPSDFFPSV-NH2		N	N	N	N	N		Amidated
13	LLSFLPSDFFPSV-NH2		N	N	N	N	N		Amidated
12	LSFLPSDFFPSV-NH2		N	N	N	N	N		Amidated
11	SFLPSDFFPSV-NH2		N	N	N	N	N		Amidated
10	FLPSDFFPSV-NH2		N	N	N	N	N		Amidated
9	LPSDFFPSV-NH2		N	N	N	N	N		Amidated
8	PSDFFPSV-NH2		N	N	N	N	N		Amidated
9	FLPSDFFPS-NH2		N	N	N	N	N		Amidated
8	FLPSDFFP-NH2		N	N	N	N	N		Amidated
7	FLPSDFF-NH2		N	N	N	N	N		Amidated
10	ALPSDFFPSV-NH2		N	N	N	N	N		
10	SLNFLGGTTV(NH2)								

HBV ANALOGS

AA	Sequence	Fixed Nomen.	A1 Motif	A2 Super Motif	A3 Super Motif	A24 Motif	B7 Super Motif	1° Anchor Fixer	Analog
									Amidated
11	FLPSDFFPSVR-NH2		N	N	N	N	N		A
9	ALFKDWEEL		N	Y	N	N	N		A
9	VLGGSRHKL		N	Y	N	N	N		A
9	KIKESFRKL		N	Y	N	N	N		A
9	ALMPLYASI		N	Y	N	N	N		A
9	FLSKQYLNL		N	Y	N	N	N		A
9	LLGSAANWI		N	Y	N	N	N		A
9	NLNNLNVSI		N	Y	N	N	N		A
9	IIKKSEQFV		N	Y	N	N	N		A
9	ALSLIVNLL		N	Y	N	N	N		A
9	RIPRTPRSV								
	237								

Table XXIII: Immunogenicity of HBV-derived peptides

Supermotif	Peptide	Sequence	Protein	XRN	Immunogenicity			
					primary	transgenic	patients	overall[1]
A2 supermotif	924.07	FLPSDFFPSV	HBV core 18	5	10/10	6/6	25/32[a]	+
	1069.06	LLVPFVQWFV	HBV env 338	5	3/4	6/9		+
	1147.13	FLLAQFTSAI	HBV pol 513	5		0/3		unk
	1090.77	YMDDVVLGV	HBV pol 538	5		9/9		+
	777.03	FLLTRILTI	HBV env 183	4			14/23[a]	+
	927.15	ALMPLYACI	HBV pol 642	4	10/12	3/5	2/15[a]	+
	1013.01	WLSLLVPFV	HBV env 335	4	2/6	5/9	23/29[a]	+
	1069.05	LLAQFTSAI	HBV pol 504	4	0/4	0/5		unk
	1132.01	LVPFVQWFV	HBV env 339	4	0/3	0/4		unk
	1147.14	VLLDYQGMLPV	HBV env 259	4	4/4	6/6		+
	927.41	LLSSNLSWL	HBV pol 992	3	0/4	0/3		unk
	927.42	NLSWLSLDV	HBV pol 411	3		2/8		+
	927.46	KLHLYSHPI	HBV pol 489	3	0/4	4/6		+
	1069.07	FLLAQFTSA	HBV pol 503	3	1/2	0/3		+
	1168.02	GLSRYVARL	HBV pol 455	3			9/13[a]	+
A2 supermotif	927.11	FLLSLGIHL	HBV pol 562	2	15/22	12/13	9/15[a]	+
	927.47	HLYSHPIIL	HBV pol 1076	2		10/14		+
	1039.03	MMWYWGPSL	HBV env 360	2	3/4	0/4		+
	1069.12	YLHTLWKAGV	HBV pol 147	2	2/4			+
	1137.02	LLDYQGMLPV	HBV env 260	2	1/2	0/4		+
	1142.07	GLLGWSPQA	HBV env 62	2	3/4	5/6		+
	1.0573	ILRGTSFVYV	HBV pol 773	1			3/7[b]	+
	1013.14	VLQAGFFLL	HBV env 177	1	0/4	5/12		+
	1069.10	LLPIFFCLWV	HBV env 378	1	3/3	0/4	2/5[c]	+
	1069.13	PLLPIFFCL	HBV env 377	1	0/4	7/12		+
	1090.06	LLVLQAGFFL	HBV env 175	1	1/5	0/4		+
	1090.12	YLVSFGVWI	HBV nuc 118	1	9/9			+
	1.0518	GLSPTVWLSV	HBV env 338	1			3/9[c]	+
	1090.14	YMDDVVLGA	HBV pol 538	1	2/7	2/5	2/7[b]	+
A3 supermotif	1147.16	HTLWKAGILYK	HBV POL 149	5	0/6	3/3	1/22	+
	1083.01	STLPETTVVRR	HBV core 141	4	3/5	6/6	8/32	+
	1150.51	GSTHVSWPK	HBV pol 398	4		3/6		+
	1.0219	FVLGGCRHK	HBV adr "X" 1550	3	0/4			unk
	1069.16	NVSIPWTHK	HBV pol 47	3	0/8	0/3	1/21	+

	1069.20	LVVDFSQFSR	HBV pol 388	3	0/4	6/6	1/22	+
	1090.10	QAFTFSPTYK	HBV pol 665	3	3/6	0/3	3/21	+
	1090.11	SAICSVVRR	HBV pol 531	3	1/4		2/22	+
A3 supermotif	1069.15	TLWKAGILYK	HBV pol 150	2	3/8	0/3	5/28	+
	1142.05	KVGNFTGLY	HBV adr POL 629	2		0/3	2/22	+
B7 supermotif	1147.05	FPHCLAFSYM	HBV POL 530	5	1/3		0/12	+
	988.05	LPSDFFPSV	HBV core 19-27	4			2/16	+
	1145.04	IPIPSSWAF	HBV ENV 313	4	0/4		1/12	+
	1147.02	HPAAMPHLL	HBV POL 429	4	0/5		0/12	unk
	1147.06	LPVCAFSSA	HBV X 58	4	1/4			+
	1147.08	YPALMPLYA	HBV POL 640	4			0/12	unk
	1145.08	FPHCLAFSYM	HBV POL 541	3	0/4			unk
B7 supermotif	1147.04	TPARVTGGVF	HBV POL 354	2			2/12	+

Immunogenicity evaluation derived from primary cultures, acute patients (a-Bertoni et al, J Clin Invest 100:503, b- Rehermann et al., J. Clin. Invest 97:1655, c- Nayersina et al., J Immunol 150:4659) or transgenic mice. A positive assessment (+) is assigned when responders have been noted in one of these systems. Unk=unknown

Table XXIV. MHC-peptide binding assays: cell lines and radiolabeled ligands.

A. Class I binding assays

Species	Antigen	Allele	Cell line	Radiolabeled peptide			Notes
				Source	Sequence	IC50 nM	
Human	A1	A*0101	Steinlin	Hu. J chain 102-110	YTAVVPLVY		no NEN in PI cocktail
	A2	A*0201	JY	HBVc 18-27 F6->Y	FLPSDYFPSV		"
	A2	A*0202	P815 (transfected)	HBVc 18-27 F6->Y	FLPSDYFPSV		"
	A2	A*0203	FUN	HBVc 18-27 F6->Y	FLPSDYFPSV		"
	A2	A*0206	CLA	HBVc 18-27 F6->Y	FLPSDYFPSV		"
	A2	A*0207	21.221 (transfecte	HBVc 18-27 F6->Y	FLPSDYFPSV		"
	A3		GM3107	non-natural (A3CON1)	KVFPYALINK		"
	A11		BVR	non-natural (A3CON1)	KVFPYALINK		"
	A24	A*2402	KAS116	non-natural (A24CON1)	AYIDNYNKF		"
	A31	A*3101	SPACH	non-natural (A3CON1)	KVFPYALINK		"
	A33	A*3301	LWAGS	non-natural (A3CON1)	KVFPYALINK		"
	A28/68	A*6801	C1R	HBVc 141-151 T7->Y	STLPETYVVRR		"
	A28/68	A*6802	AMAI	HBV pol 646-654 C4->A	FTQAGYPAL		"
	B7	B*0702	GM3107	A2 sigal seq. 5-13 (L7->Y)	APRTLVYLL		"
	B8	B*0801	Steinlin	IVgp 586-593 Y1->F, Q5->	FLKDYQLL		"
	B27	B*2705	LG2	R 60s	FRYNGLIHR		"
	B35	B*3501	C1R, BVR	non-natural (B35CON2)	FPFKYAAAF		"
	B35	B*3502	TISI	non-natural (B35CON2)	FPFKYAAAF		"
	B35	B*3503	EHM	non-natural (B35CON2)	FPFKYAAAF		"
	B44	B*4403	PITOUT	EF-1 G6->Y	AEMGKYSFY		"
	B51		KAS116	non-natural (B35CON2)	FPFKYAAAF		"
	B53	B*5301	AMAI	non-natural (B35CON2)	FPFKYAAAF		"
	B54	B*5401	KT3	non-natural (B35CON2)	FPFKYAAAF		"
	Cw4	Cw*0401	C1R	non-natural (C4CON1)	QYDDAVYKL		"
	Cw6	Cw*0602	721.221 transfecte	non-natural (C6CON1)	YRHDGGNVL		"
	Cw7	Cw*0702	721.221 transfecte	non-natural (C6CON1)	YRHDGGNVL		"
Mouse	D^b		EL4	Adenovirus E1A P7->Y	SGPSNTYPEI		"
	K^b		EL4	VSV NP 52-59	RGYVFQGL		"
	D^d		P815	HIV-IIIB ENV G4->Y	RGPYRAFVTI		"
	K^d		P815	non-natural (KdCON1)	KFNPMKTYI		"
	L^d		P815	HBVs 28-39	IPQSLDSYWTSL		"

B. Class II binding assays

Species	Antigen	Allele	Cell line	Radiolabeled peptide			Notes
				Source	Sequence	IC50 nM	
Human	DR1	DRB1*0101	LG2	HA Y307-319	YPKYVKQNTLKLAT		
	DR2	DRB1*1501	L466.1	MBP 88-102Y	VVHFFKNIVTPRTPPY		
	DR2	DRB1*1601	L242.5	non-natural (760.16)	YAAFAAAKTAAAFA		
	DR3	DRB1*0301	MAT	MT 65kD Y3-13	YKTIAFDEEARR		optimal assay pH is 4.5
	DR4w4	DRB1*0401	Preiss	non-natural (717.01)	YARFQSQTTLKQKT		
	DR4w10	DRB1*0402	YAR	non-natural (717.10)	YARFQRQTTLKAAA		
	DR4w14	DRB1*0404	BIN 40	non-natural (717.01)	YARFQSQTTLKQKT		
	DR4w15	DRB1*0405	KT3	non-natural (717.01)	YARFQSQTTLKQKT		
	DR7	DRB1*0701	Pitout	Tet. tox. 830-843	QYIKANSKFIGITE		
	DR8	DRB1*0802	OLL	Tet. tox. 830-843	QYIKANSKFIGITE		
	DR8	DRB1*0803	LUY	Tet. tox. 830-843	QYIKANSKFIGITE		
	DR9	DRB1*0901	HID	Tet. tox. 830-843	QYIKANSKFIGITE		
	DR11	DRB1*1101	Sweig	Tet. tox. 830-843	QYIKANSKFIGITE		
	DR12	DRB1*1201	Herluf	unknown eluted peptide	EALIHQLKINPYVLS		
	DR13	DRB1*1302	H0301	Tet. tox. 830-843 S->A	QYIKANAKFIGITE		
	DR51	DRB5*0101	JM3107 or L416.5	Tet. tox. 830-843	QYIKANAKFIGITE		
	DR51	DRB5*0201	L255.1	HA 307-319	PKYVKQNTLKLAT		
	DR52	DRB3*0101	MAT	Tet. tox. 830-843	NGQIGNDPNRDIL		
	DR53	DRB4*0101	L257.6	non-natural (717.01)	YARFQSQTTLKQKT		no NEM in PI mix
	DQ3.1	QA1*0301/DQB1*03(	PF	non-natural (ROIV)	AHAAHAAHAAHAAHAA		
Mouse	IA^b		DB27.4	non-natural (ROIV)	AHAAHAAHAAHAAHAA		optimal assay pH is 5.5
	IA^d		A20	non-natural (ROIV)	AHAAHAAHAAHAAHAA		
	IA^k		CH-12	HEL 46-61	YNTDGSTDYGILQINSR		optimal assay pH is 5.(
	IA^s		LS102.9	non-natural (ROIV)	AHAAHAAHAAHAAHAA		
	IA^u		91.7	non-natural (ROIV)	AHAAHAAHAAHAAHAA		
	IE^d		A20	Lambda repressor 12-26	YLEDARRKKAIYEKKK		optimal assay pH is 5.(
	IE^k		CH-12	Lambda repressor 12-26	YLEDARRKKAIYEKKK		optimal assay pH is 5.(

Table XXV. Monoclonal antibodies used in MHC purifi

Monoclonal antibody	Specificity
W6/32	HLA-class I
B123.2	HLA-B and C
IVD12	HLA-DQ
LB3.1	HLA-DR
M1/42	H-2 class I
28-14-8S	H-2 D^b and L^d
34-5-8S	H-2 D^d
B8-24-3	H-2 K^b
SF1-1.1.1	H-2 K^d
Y-3	H-2 K^b
10.3.6	H-2 IA^k
14.4.4	H-2 IE^d, IE^K
MKD6	H-2 IA^d
Y3JP	H-2 IA^b, IA^s, IA^u

Table XXVI: in vitro binding of conserved HBV-derived peptides to HLA-A2-supertype alleles.

Peptide	AA	Molecule	1st Pos	Sequence	Consv.[1]	A2-supertype binding capacity (IC50 nM)					Alleles bound[2]
						A*0201	A*0202	A*0203	A*0206	A*6802	
924 07	10	Core	18	FLPSDFFPSV	95	2 5	2 1	6 0	3 0	36	5
1069 06	10	ENV	349	LLVPFVQWFV	95	7 5	11	5 9	13	286	5
1147.13	10	POL	524	FLLAQFTSAI	95	24	134	1 4	34	455	5
1013 0102	9	ENV	346	WLSLLVPFV	100	4.6	113	1.4	10	1290	4
777 03	9	ENV	183	FLLTRILTI	80	9 8	100	1 3	19	-[3]	4
927 15	9	POL	653	ALMPLYACI	95	10	126	3 0	160	851	4
1069 05	9	POL	525	LLAQFTSAI	95	50	16	3 0	1538	51	4
1132 01	9	ENV	350	LVPFVQWFV	95	119	287	2083	463	14	4
1147 14	11	ENV	259	VLLDYQGMLPV	90	8 6	20	2 0	13	2353	4
1090 77	9	POL	538 (a)	YMDDVVLGV	90	5 1	90	6 7	71	1905	4
1069 071	9	POL	524	FLLAQFTSA	95	6 0	1654	9 1	39	870	3
927 46	9	POL	500	KLHLYSHPI	95	72	126	3 7	627	26667	3
927 42	9	POL	422	NLSWLSLDV	90	77	843	16	2313	404	3
1168 02	9	POL	455	GLSRYVARL	90	79	391	18	12333	-	3
927.41	9	POL	418	LLSSNLSWL	90	455	55	2 6	1370	4000	3
1039.031	9	ENV	360	MMWYWGPSL	85	5.6	5375	833	112	3636	2
927.11	9	POL	573	FLLSLGIHL	95	7.7	4300	1000	34	11429	2
1142.07	9	ENV	73	GLLGWSPQA	85	13	14333	286	1429	-	2
927.47	9	POL	502	HLYSHPIIL	80	23	14333	11	2176	755	2
1137.02	10	ENV	271	LLDYQGMLPV	90	51	-	500	552	-	2
1069 09	9	ENV	270	VLLDYQGML	95	114	-	476	4111	-	2
1069.14	10	NUC	168	ILSTLPETTV	100	238	506	130	1194	5970	2
1069.11	10	POL	147	YLHTLWKAGI	100	313	8600	18	4000	1250	2
1142 01	9	NUC	129	LLWFHISCL	90	385	21500	238	1194	4082	2
1090 12	9	NUC	147	YLVSFGVWI	90	13					1
1.0518	10	ENV	359	GLSPTVWLSV	75	18					1
1013 1402	9	ENV	177	VLQAGFFLL	95	33	2389	3704	1947	6349	1
1069.13	9	ENV	388	PLLPIFFCL	100	77	-	5556	3364	8511	1
1069.10	10	ENV	389	LLPIFFCLWV	100	156	5375	667	5000	-	1
1090.06	10	ENV	175	LLVLQAGFFL	90	161	1162	2222	2467	3636	1
1 0895	10	ENV	248	FILLLCLIFL	80	179					1
927.24	9	POL	770	WILRGTSFV	80	185					1
1090.14	9	POL	538	YMDDVVLGA	90	200	-	4167	-	-	1
3.0205	10	ENV	171	FLGPLLVLQA	75	263					1
1069 08	10	ENV	260	ILLLCLIFLL	100	263	-	-	2846	26667	1
1 0573	10	POL	773	ILRGTSFVYV	80	313					1

1. Frequency of entire sequence amongst isolates scanned.
2. Number of supertpe alleles bound. Peptides binding 3 or more alleles are considered degenerate.
3. A dash (-) indicates IC50

Table XXVII: in vitro binding of conserved HBV-derived peptides to HLA-A3-supertype alleles.

Peptide	AA	Molecule	1st Pos	Sequence	Consv.[1]	A3-supertype binding capacity (IC50 nM)					Alleles bound
						A*03	A*11	A*3101	A*3301	A*6801	
26 0535	11	X NUC FUS	299	GVWIRTPPAYR	95	58	35	3 0	40	12	5
1147.16	11	pol	149	HTLWKAGILYK	100	20	14	486	403	42	5
26 0539	11	POL	376	RLVVDFSQFSR	95	39	2 0	7.0	24	1 0	5
26 0149	9	X	69	CALRFTSAR	85	3235	261	12	3.6	11	4
1.0993	9	X	130	KVFVLGGCR	75	262	73	30	408	2667	4
26.0153	9	X	64	SSAGPCALR	90	1375	43	55	181	11	4
1083.01	11	Core	141	STLPETTVVRR	95	733	4 0	180	181	26	4
20.0130	9	pol	655	AFTFSPTYK	95	42	150	3103	13182	296	3
26.0008	8	POL	656	FTFSPTYK	95	193	136	1286	1000	73	3
1.0219	9	X	1550	FVLGGCRHK	80	169	316	1500	744	103	3
1069.20	10	POL	388	LVVDFSQFSR	100	6875	17	692	126	16	3
1069 16	9	POL	47	NVSIPWTHK	100	134	105	-[3]	2900	250	3
1090.10	10	POL	665	QAFTFSPTYK	95	244	11	18000	5088	6.7	3
1090.11	9	POL	531	SAICSVVRR	95	1897	29	1200	446	21	3
20.0131	9	pol	524	SVVRRAFPH	95	100	10	621	-	500	3
26.0545	11	X NUC FUS	318	TLPETTVVRRR	95	22000	375	2951	408	13	3
26 0023	8	X NUC FUS	296	VSFGVWIR	90	2750	207	240	1074	222	3
1142.05	9	POL	55	KVGNFTGLY	95	52	353	-	-	-	2
1142.06	9	POL	623	PVNRPIDWK	85	355	43	-	-	8889	2
1 0975	9	POL	106	RLKLIMPAR	75	116	-	5 8	592	-	2
1.0562	10	POL	576	SLGIHLNPNK	75	55	77				2
1069.21	10	NUC	170	STLPETTVVR	95	15714	100	2250	1208	320	2
1069.22	10	NUC	171	TLPETTVVRR	95	15714	261	-	2417	182	2
1069.15	10	POL	150	TLWKAGILYK	100	2 1	17	3529	29000	615	2
1.0215	9	X	105	TTDLEAYFK	75	18333	6.5	-	24167	471	2
1069.17	10	POL	369	VTGGVFLVDK	100	282	65	-	-	3636	2
1069.19	9	POL	389	VVDFSQFSR	100	7333	80	13846	1706	242	2
26 0026	8	POL	168	ASFCGSPY	100	239	26	-	-	20000	2
26.0549	11	ENV	389	LLPIFFCLWVY	100	478	10000	2609	644	82	2
26.0550	11	POL	528	RAFPHCLAFSY	95	92	15	667	26364	2667	2
1090 04	10	POL	746	GTDNSVVLSR	90	11000	143	6000	15263	10000	1
1069.04	10	POL	149	HTLWKAGILY	100	250	7500	-	8529	6667	1
1.0205	9	POL	771	ILRGTSFVY	80	250	-	-	-	-	1
1090.08	9	NUC	148	LVSFGVWIR	90	3929	500				1
1039 01	10	ENV	360	MMWYWGPSLY	85	220	7500	-	-	26667	1
1.0584	10	X	104	STTDLEAYFK	75	1667	2.2				1
1147.17	11	pol	735	GTDNSVVLSRK	90	786	11	-	-	-	1
1147.18	11	pol	357	RVTGGVFLVDK	100	578	207	-	-	-	1
1099.03	9	POL	150	TLWKAGILY	100	85	7500	-	-	-	1
1090.15	10	POL	549	YMDDVVLGAK	90	333	1395	-	-	-	1
26.0024	8	POL	50	VSIPWTHK	100	846	353	5806	22308	20000	1

1. Frequency of entire sequence amongst isolates scanned.
2. Number of supertpe alleles bound. Peptides binding 3 or more alleles are considered degenerate.
3. A dash (-) indicates IC50

Table XXVIII: in vitro binding of conserved HBV-derived peptides to HLA-B7 supertype alleles.

Peptide	AA	Molecule	1st Pos	Sequence	Consv [1]	B7-supertype binding capacity (IC50 nM)					Alleles bound [2]
						B*0702	B*3501	B*5101	B*5301	B*5401	
1147.05	10	POL	541	FPHCLAFSYM	95	56	33	61	118	208	5
1145.04	9	ENV	324	IPIPSSWAF	100	42	26	2.3	12	2941	4
1147.02	9	POL	440	HPAAMPHLL	100	56	267	500	186	833	4
1147.06	9	X	58	LPVCAFSSA	95	115	101	500	10333	0 53	4
1147.08	9	POL	651	YPALMPLYA	95	306	150	162	664	0.63	4
988 05	9	CORE	19	LPSDFFPSV	95	1774	343	9.0	120	4 8	4
1145.08	9	POL	541	FPHCLAFSY	95	- [3]	14	83	17	503	3
19.0014	8	POL	640	YPALMPLY	190	13750	28	13	207	1786	3
26 0570	11	pol	640	YPALMPLYACI	95	1375	-	117	291	143	3
1147.04	10	POL	365	TPARVTGGVF	90	17	72	-	939	16667	2
15 0034	9	ENV	390	LPIFFCLWV	100	-	-	57	2325	53	2
20 0140	9	POL	723	LPIHTAELL	85	1375	114	1058	30	20000	2
19 0006	8	ENV	340	VPFVQWFV	95	5500	-	0 29	-	91	2
19 0007	8	ENV	379	LPIFFCLW	100	-	-	153	66	2857	2
19 0010	8	POL	1	MPLSYQHF	100	-	742	458	251	526	2
19.0011	8	POL	429	HPAAMPHL	100	85	18000	18	2514	625	2
19.0012	8	POL	511	SPFLLAQF	95	10	8000	306	10333	1075	2
26.0566	11	pol	511	SPFLLAQFTSA	95	67	-	-	-	0.83	2
1147.01	9	POL	789	DPSRGRLGL	90	458	-	-	-	-	1
16 0182	10	X	67	GPCALRFTSA	90	61	-	-	-	2857	1
20 0273	10	POL	440	HPAAMPHLLV	85	344	3600	705	664	588	1
15.0030	9	ENV	191	IPQSLDSWW	90	-	-	27500	62	-	1
15.0210	10	POL	123	LPLDKGIKPY	100	-	248	27500	-	-	1
16 0006	9	ENV	25	FPDHQLDPA	90	-	8000	-	-	12	1
16.0177	10	ENV	324	IPIPSSWAFA	80	4231	3000		6643	22	1
16.0180	10	POL	644	APFTQCGYPA	95	1897	-		-	7.1	1
16.0181	10	POL	723	LPIHTAELLA	85	3056	6545		5813	30	1
19.0003	8	ENV	173	GPLLVLQA	95	18333	-	500	-	1538	1
19 0005	8	ENV	313	IPIPSSWA	100	13750	18000	2895	-	167	1
19.0009	8	NUC	133	RPPNAPIL	100	724	-	196	-	-	1
19 0015	8	POL	659	SPTYKAFL	95	14	-	2895	-	-	1
19 0016	8	POL	769	VPSALNPA	90	5000	-	786	-	10	1
26.0554	11	pol	633	APFTQCGYPAL	95	24	7200	13750	-	1075	1
26 0559	11	pol	712	LPIHTAELLAA	85	611	2667	-	775	3.6	1
26.0561	11	pol	774	NPADDPSRGRL	90	458	-	-	-	-	1
26 0564	11	Core	133	RPPNAPILSTL	100	42	-	3056	-	-	1
26.0567	11	Core	49	SPHHTALRQAI	100	9.5	-	13750	18600	-	1
26 0568	11	pol	354	TPARVTGGVFL	90	58	-	-	18600	20000	1

1. Frequency of entire sequence amongst isolates scanned.
2. Number of supertpe alleles bound. Peptides binding 3 or more alleles are considered degenerate.
3. A dash (-) indicates IC50

Table XXIX: HBV derived A1- and A24-motif containing peptides

a. A1-motif peptides

Peptide	Molecule	Position	Sequence	Conserv.	HLA-A*0101 binding (IC50 nM)
1069.01	Core	59	LLDTASALY	75	2.1
1.0519	Core	419	DLLDTASALY	75	2.3
1069.02	pol	427	SLDVSAAFY	95	4.8
2.0239		1000	LSLDVSAAFY	95	6.0
2.0126		1521	MSTTDLEAY	75	29
1039.06	ENV	359	WMMWYWGPSLY	85	78
1090.14	pol	538	YMDDVVLGA	90	96
1090.09	pol	808	PTTGRTSLY	85	119
1069.03	pol	124	PLDKGIKPYY	100	147
1069.08	env	249	ILLLCLIFLL	100	192
1069.04	pol	149	HTLWKAGILY	100	381
1039.01		360	MMWYWGPSLY	85	309
1.0774	Core	416	WLWGMDIDPY	75	309
20.0254	pol	631	FAAPFTQCGY	95	368
1.0166	pol	629	KVGNFTGLY	95	368

A dash indicates IC50 nM

b. A24 -motif peptides

Peptide	Molecule	Position	Sequence	Conserv.	HLA-A*2402 binding (IC50 nM)
20.0271.	POL	392	SWPKFAVPNL	95	2.1
1069.23	POL	745	KYTSFPWLL	85	2.3
2.0181	POL	492	LYSHPIILGF	80	11
20.0269	ENV	236	RWMCLRRFII	95	11
20.0136	ENV	334	SWLSLLVPF	100	31
20.0137	ENV	197	SWWTSLNFL	95	32
20.0135	ENV	236	RWMCLRRFI	95	169
20.0139	POL	167	SFCGSPYSW	100	169
2.0173	POL	4	SYQHFRKLLL	75	182
2.0060		1224	GYPALMPLY	95	245
13.0129	NUC	117	EYLVSFGVWI	90	353
1090.02	core	131	AYRPPNAPI	90	387
13.0073	NUC	102	WFHISCLTF	80	400
20.0138	POL	51	PWTHKVGNF	100	414

A dash indicates IC50 nM

Table XXXa: Immunogenicity of HBV-derived A2-supermotif cross-reactive peptides

Peptide	Sequence	Protein	XRN	Immunogenicity			
				primary	transgenic	patients	overall[1]
924.07	FLPSDFFPSV	HBV core 18	5	10/10	6/6	25/32[a]	+
1069.06	LLVPFVQWFV	HBV env 338	5	3/4	6/9		+
1147.13	FLLAQFTSAI	HBV pol 513	5		0/3		-
1090.77	YMDDVVLGV	HBV pol 538	5		9/9		+
777.03	FLLTRILTI	HBV env 183	4			14/23[a]	+
927.15	ALMPLYACI	HBV pol 642	4	10/12	3/5	2/15[a]	+
1013.01	WLSLLVPFV	HBV env 335	4	2/6	5/9	23/29[a]	+
1069.05	LLAQFTSAI	HBV pol 504	4	0/4	0/5		-
1132.01	LVPFVQWFV	HBV env 339	4	0/3	0/4		-
1147.14	VLLDYQGMLPV	HBV env 259	4	4/4	6/6		+
927.41	LLSSNLSWL	HBV pol 992	3	0/4	0/3		-
927.42	NLSWLSLDV	HBV pol 411	3		2/8		+
927.46	KLHLYSHPI	HBV pol 489	3	0/4	4/6		+
1069.07	FLLAQFTSA	HBV pol 503	3	1/2	0/3		+
1168.02	GLSRYVARL	HBV pol 455	3			9/13[a]	+

Immunogenicity evaluation derived from primary cultures, acute patients (a-Bertoni et al, J Clin Invest 100:503, b- Rehermann et al., J. Clin. Invest 97:1655, c- Nayersina et al., J Immunol 150:4659) or transgenic mice. A positive assessment (+) is assigned when responders have been noted in one of these systems.

Table XXXb: Immunogenicity of non-crossreactive HBV A2-supermotif peptides

Peptide	Sequence	Protein	XRN	Immunogenicity			
				primary	transgenic	patients	overall[1]
927.11	FLLSLGIHL	HBV pol 562	2	15/22	12/13	9/15[a]	+
927.47	HLYSHPIIL	HBV pol 1076	2		10/14		+
1039.03	MMWYWGPSL	HBV env 360	2	3/4	0/4		+
1069.12	YLHTLWKAGV	HBV pol 147	2	2/4			+
1137.02	LLDYQGMLPV	HBV env 260	2	1/2	0/4		+
1142.07	GLLGWSPQA	HBV env 62	2	3/4	5/6		+
1.0573	ILRGTSFVYV	HBV pol 773	1			3/7[b]	+
1013.14	VLQAGFFLL	HBV env 177	1	0/4	5/12		+
1069.10	LLPIFFCLWV	HBV env 378	1	3/3	0/4	2/5[c]	+
1069.13	PLLPIFFCL	HBV env 377	1	0/4	7/12		+
1090.06	LLVLQAGFFL	HBV env 175	1	1/5	0/4		+
1090.12	YLVSFGVWI	HBV nuc 118	1	9/9			+
1.0518	GLSPTVWLSV	HBV env 338	1			3/9[c]	+
1090.14	YMDDVVLGA	HBV pol 538	1	2/7	2/5	2/7[b]	+

Immunogenicity evaluation derived from primary cultures, acute patients (a-Bertoni et al, J Clin Invest 100:503, b- Rehermann et al., J. Clin. Invest 97:1655, c- Nayersina et al., J Immunol 150:4659) or transgenic mice. A positive assessment (+) is assigned when responders have been noted in one of these systems.

Table XXXc: Cross-recognition of HBV pol 538 and a Lamivudine induced pol 538 variant by CTL induced with a pol 538 analog[a].

	Day 6 CTL response (ΔLU)	
Stimulating peptide	HBV pol 538 (YMDDVVLGA)[b]	HBV pol 538 mutant (YVDDVVLGA)
HBV pol 538	27.8	54.2
HBV pol 538 mutant	35.3	27.9

a. CTLs were induced using the 1090.77 analog of HBV pol 538 (peptide 1090.14). 1090.77 was encoded in the DNA minigene pEP2.AOS.

b. Values shown represent the geometric mean of ΔLU from 2 independent cultures. Peptides loaded onto target cells were 1090.14 (HBV pol 538) or 1353.02 (a Lamivudine induced mutant of pol 538).

Table XXXIa: Immunogenicity of HBV-derived A3-supermotif cross-reactive peptides

Peptide	Sequence	Protein	XRN	Immunogenicity			
				primary	transgenic	patients	overall[1]
1147.16	HTLWKAGILYK	HBV POL 149	5	0/6	3/3	1/22	+
1083.01	STLPETTVVRR	HBV core 141	4	3/5	6/6	8/32	+
1150.51	GSTHVSWPK	HBV pol 398	4		3/6		+
1.0219	FVLGGCRHK	HBV adr "X" 1550	3	0/4			-
1069.16	NVSIPWTHK	HBV pol 47	3	0/8	0/3	1/21	+
1069.20	LVVDFSQFSR	HBV pol 388	3	0/4	6/6	1/22	+
1090.10	QAFTFSPTYK	HBV pol 665	3	3/6	0/3	3/21	+
1090.11	SAICSVVRR	HBV pol 531	3	1/4		2/22	+

1. Immunogenicity evaluation derived from primary cultures, Bertoni et al, J Clin Invest 100:503 or transgenic mice. A positive assessment (+) is assigned when responders have been noted in one of these systems. A negative assessment (-) indicates that no responders when examined.

Table XXXIb: Immunogenicity of non-crossreactive HBV A3-supermotif peptides

Peptide	Sequence	Protein	XRN	Immunogenicity			
				primary	transgenic	patients	overall[1]
1069.15	TLWKAGILYK	HBV pol 150	2	3/8	0/3	5/28	+
1142.05	KVGNFTGLY	HBV adr POL 629	2		0/3	2/22	+

1. Immunogenicity evaluation derived from primary cultures, Bertoni et al, J Clin Invest 100:503 or transgenic mice. A positive assessment (+) is assigned when responders have been noted in one of these systems. A negative assessment (-) indicates that no responders when examined.

Table XXXIIa: Immunogenicity of HBV B7-supermotif cross-reactive peptides

Peptide	Sequence	Protein	XRN	Immunogenicity			
				primary	transgenic	patients	overall[1]
1147.05	FPHCLAFSYM	HBV POL 530	5	1/3		0/12	+
988.05	LPSDFFPSV	HBV core 19-27	4			2/16	+
1145.04	IPIPSSWAF	HBV ENV 313	4	0/4		1/12	+
1147.02	HPAAMPHLL	HBV POL 429	4	0/5		0/12	-
1147.06	LPVCAFSSA	HBV X 58	4	1/4			+
1147.08	YPALMPLYA	HBV POL 640	4			0/12	-
1145.08	FPHCLAFSY	HBV POL 541	3	0/4			-

1. Immunogenicity evaluation derived from primary cultures, Bertoni et al, J Clin Invest 100:503 or transgenic mice. A positive assessment (+) is assigned when responders have been noted in one of these systems. A negative assessment (-) indicates that no responders when examined.

Table XXXIIb: Immunogenicity of non-crossreactive HBV B7-supermotif peptides

Peptide	Sequence	Protein	XRN	Immunogenicity			
				primary	transgenic	patients	overall[1]
1147.04	TPARVTGGVF	HBV POL 354	2			2/12	+

1. Immunogenicity evaluation derived from primary cultures, Bertoni et al, J Clin Invest 100:503 or transgenic mice. A positive assessment (+) is assigned when responders have been noted in one of these systems. A negative assessment (-) indicates that no responders when examined.

Table XXXIII. Candidate HBV-derived HTL epitopes

Selection criteria	Peptide	Mol	1st Pos	Conservancy		Sequence
				Core	Total	
DR-supermotif	F107.01	ENV	249	100	95	ILLLCLIFLLVLLDY
	F107.02	ENV	252	95	95	LCLIFLLVLLDYQGM
	1280.17	ENV	258	90	90	LVLLDYQGMLPVCPL
	1186.22	ENV	332	100	100	RFSWLSLLVPFVQWF
	1186.15	ENV	339	95	95	LVPFVQWFVGLSPTV
	1186.06	ENV	342	95	95	FVQWFVGLSPTVWLS
	1186.03	NUC	19	85	85	ASKLCLGWLWGMDID
	1186.12	NUC	24	85	85	LGWLWGMDIDPYKEF
	857.02	NUC	50		90	PHHTALRQAILCWGELMTLA
	1186.23	NUC	98	85	85	RQLLWFHISCLTFGR
	27.0279	NUC	117		90	EYLVSFGVWIRTPPA
	27.0280	NUC	123	95	95	GVWIRTPPAYRPPNA
	1186.20	NUC	129	100	95	PPAYRPPNAPILSTL
	1186.16	NUC	136	100	95	NAPILSTLPETTVVR
	1186.01	POL	38	95	95	AEDLNLGNLNVSIPW
	1186.17	POL	45	100	95	NLNVSIPWTHKVGNF
	27.0281	POL	145	100	100	RHYLHTLWKAGILYK
	1280.13	POL	406	95	95	KFAVPNLQSLTNLLS
	27.0283	POL	409		85	VPNLQSLTNLLSSNL
	F107.03	POL	412	90	90	LQSLTNLLSSNLSWL
	1186.28	POL	416	90	90	TNLLSSNLSWLSLDV
	1186.27	POL	420	100	85	SSNLSWLSLDVSAAF
	F107.04	POL	523	95	95	PFLLAQFTSAICSVV
	1186.10	POL	526	95	95	LAQFTSAICSVVRRA
	1186.04	POL	534	95	95	CSVVRRAFPHCLAFS
	F107.05	POL	538	95	95	RRAFPHCLAFSYMDD
	1186.02	POL	546	90	90	AFSYMDDVVLGAKSV
	1186.05	POL	629	85	85	DWKVCQRIVGLLGFA
	1280.21	POL	637	95	95	VGLLGFAAPFTQCGY
	27.0278	POL	643		95	AAPFTQCGYPALMPL
	1186.21	POL	648	95	95	QCGYPALMPLYACIQ
	1280.14	POL	694	95	95	LCQVFADATPTGWGL
	27.0282	POL	750	85	85	SVVLSRKYTSFPWLL
		X	13	95	90	RDVLCLRPVGAESRG
	1186.07	X	50	95	90	GAHLSLRGLPVCAFS
	1186.29	X	60	95	90	VCAFSSAGPCALRFT
Algorithm	1280.20	ENV	330	100	80	SVRFSWLSLLVPFVQ
	1280.19	NUC	28	85	80	RDLLDTASALYREAL
	1298.02	POL	56	90	55	VGNFTGLYSSTVPVF
	1298.03	POL	571	95	75	TNFLLSLGIHLNPNK
	1298.05	POL	651	95	55	YPALMPLYACIQSKQ
	1298.06	POL	664	95	60	KQAFTFSPTYKAFLC
	1280.181	POL	722	85	80	PLPIHTAELLAACFA
	1280.09	POL	774	90	80	GTSFVYVPSALNPAD
DR3-motif	795.05	ENV	10		95	PLGFFPDHQLDP
	35.0090	ENV	312	95	90	FLLVLLDYQGMLPVC
	CF-03	NUC	28	85	80	RDLLDTASALYREALESPEH
	35.0091	POL	18	90	65	AGPLEEELPRLADEG
	35.0092	POL	34	100	85	NRRVAEDLNLGNLNV
	35.0093	POL	96	85	60	VGPLTVNEKRRLKLI
	35.0094	POL	120	100	100	TKYLPLDKGIKPYYP

Table XXXIII. Candidate HBV-derived HTL epitopes

Selection criteria	Peptide	Mol	1st Pos	Conservancy		Sequence
				Core	Total	
DR-supermotif	F107.01	ENV	249	100	95	ILLLCLIFLLVLLDY
	F107.02	ENV	252	95	95	LCLIFLLVLLDYQGM
	1280.17	ENV	258	90	90	LVLLDYQGMLPVCPL
	1186.22	ENV	332	100	100	RFSWLSLLVPFVQWF
	1186.15	ENV	339	95	95	LVPFVQWFVGLSPTV
	1186.06	ENV	342	95	95	FVQWFVGLSPTVWLS
	1186.03	NUC	19	85	85	ASKLCLGWLWGMDID
	1186.12	NUC	24	85	85	LGWLWGMDIDPYKEF
	857.02	NUC	50		90	PHHTALRQAILCWGELMTLA
	1186.23	NUC	98	85	85	RQLLWFHISCLTFGR
	27.0279	NUC	117		90	EYLVSFGVWIRTPPA
	27.0280	NUC	123	95	95	GVWIRTPPAYRPPNA
	1186.20	NUC	129	100	95	PPAYRPPNAPILSTL
	1186.16	NUC	136	100	95	NAPILSTLPETTVVR
	1186.01	POL	38	95	95	AEDLNLGNLNVSIPW
	1186.17	POL	45	100	95	NLNVSIPWTHKVGNF
	27.0281	POL	145	100	100	RHYLHTLWKAGILYK
	1280.13	POL	406	95	95	KFAVPNLQSLTNLLS
	27.0283	POL	409		85	VPNLQSLTNLLSSNL
	F107.03	POL	412	90	90	LQSLTNLLSSNLSWL
	1186.28	POL	416	90	90	TNLLSSNLSWLSLDV
	1186.27	POL	420	100	85	SSNLSWLSLDVSAAF
	F107.04	POL	523	95	95	PFLLAQFTSAICSVV
	1186.10	POL	526	95	95	LAQFTSAICSVVRRA
	1186.04	POL	534	95	95	CSVVRRAFPHCLAFS
	F107.05	POL	538	95	95	RRAFPHCLAFSYMDD
	1186.02	POL	546	90	90	AFSYMDDVVLGAKSV
	1186.05	POL	629	85	85	DWKVCQRIVGLLGFA
	1280.21	POL	637	95	95	VGLLGFAAPFTQCGY
	27.0278	POL	643		95	AAPFTQCGYPALMPL
	1186.21	POL	648	95	95	QCGYPALMPLYACIQ
	1280.14	POL	694	95	95	LCQVFADATPTGWGL
	27.0282	POL	750	85	85	SVVLSRKYTSFPWLL
		X	13	95	90	RDVLCLRPVGAESRG
	1186.07	X	50	95	90	GAHLSLRGLPVCAFS
	1186.29	X	60	95	90	VCAFSSAGPCALRFT
Algorithm	1280.20	ENV	330	100	80	SVRFSWLSLLVPFVQ
	1280.19	NUC	28	85	80	RDLLDTASALYREAL
	1298.02	POL	56	90	55	VGNFTGLYSSTVPVF
	1298.03	POL	571	95	75	TNFLLSLGIHLNPNK
	1298.05	POL	651	95	55	YPALMPLYACIQSKQ
	1298.06	POL	664	95	60	KQAFTFSPTYKAFLC
	1280.181	POL	722	85	80	PLPIHTAELLAACFA
	1280.09	POL	774	90	80	GTSFVYVPSALNPAD
DR3-motif	795.05	ENV	10		95	PLGFFPDHQLDP
	35.0090	ENV	312	95	90	FLLVLLDYQGMLPVC
	CF-03	NUC	28	85	80	RDLLDTASALYREALESPEH
	35.0091	POL	18	90	65	AGPLEEELPRLADEG
	35.0092	POL	34	100	85	NRRVAEDLNLGNLNV
	35.0093	POL	96	85	60	VGPLTVNEKRRLKLI
	35.0094	POL	120	100	100	TKYLPLDKGIKPYYP

Table XXXIII. Candidate HBV-derived HTL epitopes

Selection criteria	Peptide	Mol	1st Pos	Conservancy		Sequence
				Core	Total	
	35.0095	POL	371	100	55	GGVFLVDKNPHNTTE
	35.0096	POL	385	100	45	ESRLVVDFSQFSRGN
	1186.18	POL	422	95	85	NLSWLSLDVSAAFYH
	35.0099	POL	666	95	55	AFTFSPTYKAFLCKQ
	35.0101	X	18	95	35	LRPVGAESRGRPVSG
Lower conservancy or miscellaneous	799.01	ENV	11	80	75	PLLVLQAGFFLLTRILTIPQ
	799.02	ENV	31	95		SLDSWWTSLNFLGGTTVCLG
	799.04	ENV	71	95	75	GYRWMCLRRFIIFLFILLLC
	1298.01	ENV	117	80	40	PQAMQWNSTTFHQTL
	1280.06	ENV	180	80	80	AGFFLLTRILTIPQS
	1280.11	ENV	245	80	80	IFLFILLLCLIFLLV
	CF-08	NUC	120		90	VSFGVWIRTPPAYRPPNAPI
	1186.25	NUC	121	95	90	SFGVWIRTPPAYRPP
	1280.15	POL	501	80	80	LHLYSHPIILGFRKI
	1298.04	POL	618	80	45	KQCFRKLPVNRPIDW
	1298.07	POL	767	80	70	AANWILRGTSFVYVP
	1298.08	POL	827	80	60	PDRVHFASPLHVAWR

Table XXXIV. HLA-DR screening panels

Screening Panel	Antigen	Alleles	Representative Assay		Phenotypic Frequencies					
			Allele	Alias	Cauc.	Blk.	Jpn.	Chn.	Hisp.	Avg.
Primary	DR1	DRB1*0101-03	DRB1*0101	(DR1)	18.5	8.4	10.7	4.5	10.1	10.4
	DR4	DRB1*0401-12	DRB1*0401	(DR4w4)	23.6	6.1	40.4	21.9	29.8	24.4
	DR7	DRB1*0701-02	DRB1*0701	(DR7)	26.2	11.1	1.0	15.0	16.6	14.0
	Panel total				59.6	24.5	49.3	38.7	51.1	44.6
Secondary	DR2	DRB1*1501-03	DRB1*1501	(DR2w2 ß1)	19.9	14.8	30.9	22.0	15.0	20.5
	DR2	DRB5*0101	DRB5*0101	(DR2w2 ß2)	-	-	-	-	-	-
	DR9	DRB1*09011,09012	DRB1*0901	(DR9)	3.6	4.7	24.5	19.9	6.7	11.9
	DR13	DRB1*1301-06	DRB1*1302	(DR6w19)	21.7	16.5	14.6	12.2	10.5	15.1
	Panel total				42.0	33.9	61.0	48.9	30.5	43.2
Tertiary	DR4	DRB1*0405	DRB1*0405	(DR4w15)	-	-	-	-	-	-
	DR8	DRB1*0801-5	DRB1*0802	(DR8w2)	5.5	10.9	25.0	10.7	23.3	15.1
	DR11	DRB1*1101-05	DRB1*1101	(DR5w11)	17.0	18.0	4.9	19.4	18.1	15.5
	Panel total				22.0	27.8	29.2	29.0	39.0	29.4
Quarternary	DR3	DRB1*0301-2	DRB1*0301	(DR3w17)	17.7	19.5	0.4	7.3	14.4	11.9
	DR12	DRB1*1201-02	DRB1*1201	(DR5w12)	2.8	5.5	13.1	17.6	5.7	8.9
	Panel total				20.2	24.4	13.5	24.2	19.7	20.4

Table XXXV. HBV-derived cross-reactive HLA-DR binding peptides

Peptide	Mol	1st Pos	Conservancy Core	Conservancy Total	Sequence	HLA-DR binding capacity (IC50 nM) DR1	DR2w2 β1	DR2w2 β2	DR3	DR4w4	DR4w15	DR5w11	DR6	DR7	DR8	DR9	Total DR alleles bound
F107 03	POL	412	90	90	LQSLTNLLSSNLSWL	2.0	21	1000	-[a]	9 4	47	294	135	167	557	682	10
1298 06	POL	664	95	60	KQAFTFSPTYKAFLC	9 4	38	143	-	41	173	83	175	76	408	139	10
1280.06	ENV	180	80	80	AGFFLLTRILTIPQS	1.1	217	1053	-	8 5	253	5.6	9.5	8.1	188	58	9
1280.09	POL	774	90	80	GTSFVYVPSALNPAD	14	650	400	-	118	93	426	-	93	803	221	9
1186 25	NUC	121	95	90	SFGVWIRTPPAYRPP	532	827	47	-	577	603	769	17500	1042	196	938	8
27.0280	NUC	123	95	95	GVWIRTPPAYRPPNA	14	217	2 8	-	13	67	42	-	114	92	1667	8
CF-08	NUC	120		90	VSFGVWIRTPPAYRPPNAPI	192		105		300		426		124			5
27.0281	POL	145	100	100	RHYLHTLWKAGILYK	17	5 4	35	-	2250	1462	42	745	61	27	174	8
1186.15	ENV	339	95	95	LVPFVQWFVGLSPTV	385	13	1429	-	300	27	53	1944	2717	74	30	7
1280 15	POL	501	80	80	LHLYSHPIILGFRKI	227	268	500	-	66	238	488	17500	-	803	1531	7
F107 04	POL	523	95	95	PFLLAQFTSAICSVV	28	337	4762	-	563	317	1667	44	325	845	1271	7
1298.04	POL	618	80	45	KQCFRKLPVNRPIDW	3 3	4136	952	-	38	45	1538	814	63	845	3000	7
1298.07	POL	767	80	70	AANWILRGTSFVYVP	54	379	3279	-	882	1520	1429	140	43	196	278	7
857 02	NUC	50		90	PHHTALRQAILCWGELMTLA	70	9 1	211	-	85		263	193000	676	196	2273	7

a. A dash (-) indicates IC50 nM >20,000.

Table XXXVI. HBV-derived DR3-binding peptides

Peptide	Mol	1st Pos	Conservancy		Sequence	DR3
			Core	Total		
1280.14*	POL	694	95	95	LCQVFADATPTGWGL	67
35.0096	POL	385	100	45	ESRLVVDFSQFSRGN	115
35.0093	POL	96	85	60	VGPLTVNEKRRLKLI	136
1186.27	POL	420	100	85	SSNLSWLSLDVSAAF	200
1186.18	POL	422	95	85	NLSWLSLDVSAAFYH	231

*tested as peptide 35.0100

Table XXXVIIa: HBV Preferred CTL Epitopes

Peptide	Sequence	Protein	HLA
924.07	FLPSDFFPSV	core 18	A2
777.03	FLLTRILTI	env 183	A2
927.15	ALMPLYACI	pol 642	A2
1013.01	WLSLLVPFV	env 335	A2
1090.77	YMDDVVLGV	pol 538	A2/A1
1168.02	GLSRYVARL	pol 455	A2
927.11	FLLSLGIHL	pol 562	A2
1069.10	LLPIFFCLWV	env 378	A2
1069.06	LLVPFVQWFV	env 338	A2
1147.16	HTLWKAGILYK	pol 149	A3/A1
1083.01	STLPETTVVRR	core 141	A3
1069.16	NVSIPWTHK	pol 47	A3
1069.20	LVVDFSQFSR	pol 388	A3
1090.10	QAFTFSPTYK	pol 665	A3
1090.11	SAICSVVRR	pol 531	A3
1142.05	KVGNFTGLY	pol 629	A3/A1
1147.05	FPHCLAFSYM	pol 530	B7
988.05	LPSDFFPSV	core 19	B7
1145.04	IPIPSSWAF	env 313	B7
1147.02	HPAAMPHLL	pol 429	B7
26.0570	YPALMPLYACI	pol 640	B7
1147.04	TPARVTGGVF	pol 354	B7
1.0519	DLLDTASALY	core 419	A1
2.0239	LSLDVSAAFY	pol 1000	A1
1039.06	WMMWYWGPSLY	env 359	A1
20.0269	RWMCLRRFII	env 236	A24
20.0136	SWLSLLVPF	env 334	A24
20.0137	SWWTSLNFL	env 197	A24
13.0129	EYLVSFGVWI	core 117	A24
1090.02	AYRPPNAPI	core 131	A24
13.0073	WFHISCLTF	core 102	A24
20.0271	SWPKFAVPNL	pol 392	A24
1069.23	KYTSFPWLL	pol 745	A24
2.0181	LYSHPIILGF	pol 492	A24

Table XXXVIIb: HBV Preferred HTL epitopes

Selection Criteria	Peptide	Mol	1st Pos	Conservancy		Sequence
				Core	Total	
DR supermotif	F107.03	POL	412	90	90	LQSLTNLLSSNLSWL
	1298.06	POL	664	95	60	KQAFTFSPTYKAFLC
	1280.06	ENV	180	80	80	AGFFLLTRILTIPQS
	1280.09	POL	774	90	80	GTSFVYVPSALNPAD
	CF-08	CORE	120		90	VSFGVWIRTPPAYRPPNAPI
	27.0281	POL	145	100	100	RHYLHTLWKAGILYK
	1186.15	ENV	339	95	95	LVPFVQWFVGLSPTV
	1280.15	POL	501	80	80	LHLYSHPIILGFRKI
	F107.04	POL	523	95	95	PFLLAQFTSAICSVV
	1298.04	POL	618	80	45	KQCFRKLPVNRPIDW
	1298.07	POL	767	80	70	AANWILRGTSFVYVP
	857.02	CORE	50		90	PHHTALRQAILCWGELMTLA
DR3 motif	1280.14	POL	694	95	95	LCQVFADATPTGWGL
	35.0096	POL	385	100	45	ESRLVVDFSQFSRGN
	35.0093	POL	96	85	60	VGPLTVNEKRRLKLI
	1186.27	POL	420	100	85	SSNLSWLSLDVSAAF

Table XXXVIII. Estimated population coverage by a panel of HBV derived HTL epitopes

Antigen	Alleles	Representative assay	No. of epitopes[2]	Population coverage (phenotypic frequency)					
				Cauc.	Blk.	Jpn.	Chn.	Hisp.	Avg.
DR1	DRB1*0101-03	DR1	12	18.5	8.4	10.7	4.5	10.1	10.4
DR2	DRB1*1501-03	DR2w2 ß1	11	19.9	14.8	30.9	22.0	15.0	20.5
DR2	DRB5*0101	DR2w2 ß2	8	-	-	-	-	-	-
DR3	DRB1*0301-2	DR3	4	17.7	19.5	0.40	7.3	14.4	11.9
DR4	DRB1*0401-12	DR4w4	11	23.6	6.1	40.4	21.9	29.8	24.4
DR4	DRB1*0401-12	DR4w15	9	-	-	-	-	-	-
DR7	DRB1*0701-02	DR7	9	26.2	11.1	1.0	15.0	16.6	14.0
DR8	DRB1*0801-5	DR8w2	7	5.5	10.9	25.0	10.7	23.3	15.1
DR9	DRB1*09011,09012	DR9	10	3.6	4.7	24.5	19.9	6.7	11.9
DR11	DRB1*1101-05	DR5w11	11	17.0	18.0	4.9	19.4	18.1	15.5
DR13	DRB1*1301-06	DR6w19	7	21.7	16.5	14.6	12.2	10.5	15.1
Total[1]				98.5	95.1	97.1	91.3	94.3	95.1

1. Total opulation coverage has been adjusted to acount for the presence of DRX in many ethnic populations. It has been assumed that the range of specificities represented by DRX alleles will mirror those of previously characterized HLA-DR alleles. The proportion of DRX incorporated under each motif is representative of the frequency of the motif in the remainder of the population. Total coverage has not been adjusted to account for unknown gene types.

2. Number of epitopes represents a minimal estimate, considering only the epitopes shown in Table 12. Additional alleles possibly bound by nested epitopes have not been accounted.

WHAT IS CLAIMED IS:

1. A peptide composition of less than 250 amino acid residues comprising a peptide epitope useful for inducing an immune response against hepatitis B virus (HBV) said epitope (a) having an amino acid sequence of about 8 to about 13 amino acid residues that have at least 65% identity with a native amino acid sequence of HBV and, (b) binding to at least one HLA class I HLA allele with an IC_{50} of less than about 500 nM.

2. The composition of claim 1, further wherein said peptide has at least 77% identity with a native HBV amino acid sequence.

3. The composition of claim 1, further wherein said peptide has 100% identity with a native HBV amino acid sequence.

4. A pharmaceutical composition comprising a peptide and a pharmaceutical carrier, wherein the peptide is a peptide of Table VII (A1 supermotif), Table VIII (A2 supermotif/A2.1 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), or Table XVIII (A24 motif) comprising an IC_{50} of less than about 500 nM for at least one HLA class I molecule.

5. The pharmaceutical composition of claim 4 wherein the composition comprises the peptide in a form of nucleic acids that encode the peptide.

6. The pharmaceutical composition of claim 5 wherein the composition comprises the peptide in a form of nucleic acids that encode the epitope and one or more additional peptide(s).

7. The composition of claim 4, wherein the peptide is comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

8. The pharmaceutical composition of claim 4 wherein the peptide is in a human dose form, and the carrier is in a human unit dose.

9. A peptide composition of claim 1 comprising an analog of a peptide epitope, wherein the peptide epitope is an epitope of Table VII (A1 supermotif), Table VIII (A2 supermotif/A2.1 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), or Table XVIII (A24 motif), said analog comprising a preferred or less preferred amino acid of Table II substituted in for a starting residue, or having a deleterious residue of Table II substituted out of the starting sequence and replaced by a non-deleterious residue.

10. A peptide composition of claim 1 comprising a peptide of Table XXII.

11. A method for inducing a cytotoxic T lymphocyte response, said method comprising steps of:

providing a peptide that comprises an IC_{50} of less than about 500 nM for an HLA class I molecule, wherein the peptide is a peptide of Table VII (A1 supermotif), Table VIII (A2 supermotif/A2.1 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), or Table XVIII (A24 motif); and,

administering said peptide to a human.

12. The method of claim 11, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

13. The method of claim 12, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

14. The method of claim 11, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

15. A method for inducing a cytotoxic T lymphocyte response, said method comprising steps of:

providing a peptide that induces a cytotoxic T cell response *in vitro* and/or *in vivo*, wherein the peptide is a peptide of Table VII (A1 supermotif), Table VIII (A2 supermotif/A2.1 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), Table XVIII (A24 motif) or Table XXIII; and,

administering said pharmaceutical composition to a human.

16. The method of claim 15, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

17. The method of claim 16, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

18. The method of claim 15, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

19. The method of claim 15, wherein the providing step comprises a peptide that induces a cytotoxic T cell response when complexed with an HLA class I molecule and is presented to an HLA class I-restricted cytotoxic T cell.

20. A peptide composition of less than 250 amino acid residues comprising a peptide epitope useful for inducing an immune response against hepatitis B virus (HBV) said epitope (a) having an amino acid sequence of about 6 to about 25 amino acid residues that have at least 65% identity with a native amino acid sequence of HBV

and, (b) binding to at least one HLA class II HLA allele with an IC_{50} of less than about 1000 nM.

21. The composition of claim 20, further wherein said peptide has at least 77% identity with a native HBV amino acid sequence.

22. The composition of claim 20, further wherein said peptide has 100% identity with a native HBV amino acid sequence.

23. A pharmaceutical composition comprising:

a human dose form of a peptide of Table XIX or Table XX that comprises an IC_{50} of less than about 1,000 nM for at least one HLA DR molecule of an HLA DR supertype; and,

a human dose of a pharmaceutically acceptable carrier.

24. The pharmaceutical composition of claim 23 wherein the composition comprises the peptide in a form of nucleic acids that encode the peptide.

25. The pharmaceutical composition of claim 24 wherein the composition comprises the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

26. The composition of claim 25, wherein the peptide is comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

27. A peptide composition of claim 20 comprising an analog of a peptide epitope of Table XIX or Table XX, said analog comprising a preferred or less preferred amino acid of Table III substituted in for a starting residue, and/or having a deleterious residue of Table III substituted out of the starting sequence and replaced by a non-deleterious residue.

28. A method for inducing a helper T lymphocyte response, said method comprising steps of:

providing a peptide that comprises an IC_{50} of less than about 1,000 nM for an HLA class II molecule, wherein the peptide is a peptide of Table XIX or Table XX; and,

administering said peptide to a human.

29. The method of claim 28, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

30. The method of claim 29, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

31. The method of claim 28, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

32. A method for inducing a helper T lymphocyte response, said method comprising steps of:

providing a peptide that induces a helper T cell response *in vitro* and/or *in vivo*, wherein the peptide is a peptide of Table XIX or Table XX; and,

administering said pharmaceutical composition to a human.

33. The method of claim 32, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

34. The method of claim 33, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

35. The method of claim 32, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

36. The method of claim 32, wherein the providing step comprises a peptide that induces a helper T cell response when complexed with an HLA class II molecule and is presented to an HLA class I-restricted helper T cell.

37. A vaccine for preventing or treating HBV infection that induces a protective or therapeutic immune response, wherein said vaccine comprises:

at least one peptide selected from Table(s) VII-XX or Table XXII; and,

a pharmaceutically acceptable carrier.

38. A kit for a vaccine that induces a protective or therapeutic immune response to HBV, said vaccine comprising:

at least one peptide selected from Table(s) VII-XX or Table XXII;

a pharmaceutically acceptable carrier; and,

instructions for administration to a patient.

39. A method for monitoring or evaluating an immune response to HBV or an epitope thereof in a patient having a known HLA type, the method comprising:

incubating a T lymphocyte sample from the patient with a peptide selected from Table(s) VII-XX or Table XXII, wherein that peptide bears a motif corresponding to at least one HLA allele present in said patient; and,

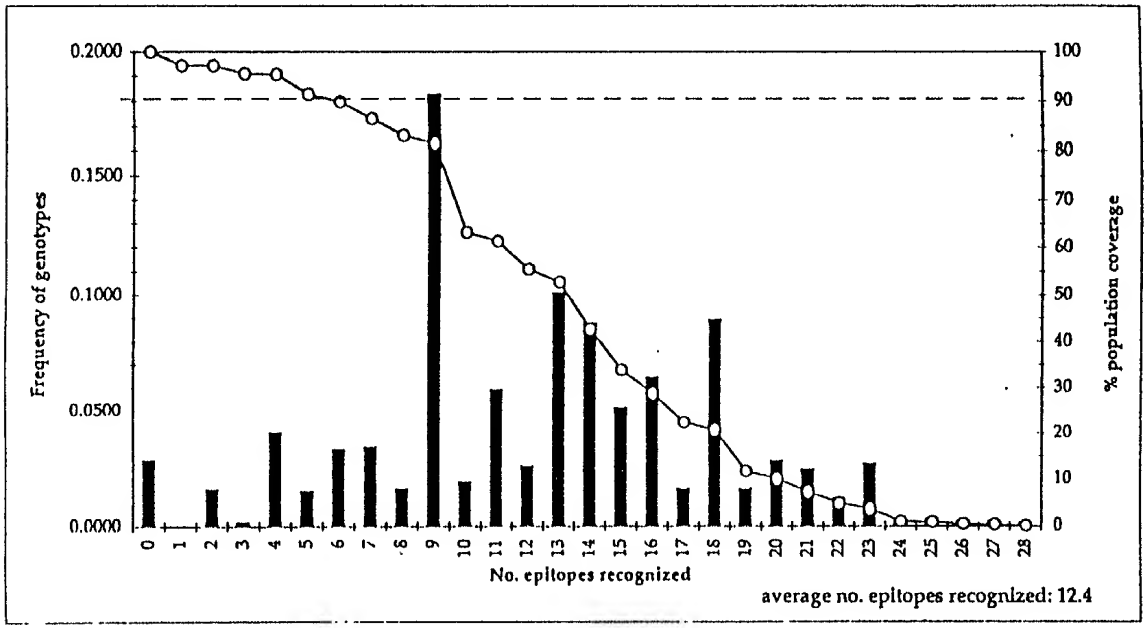
detecting the presence of a T lymphocyte that recognizes the peptide.

40. The method of claim 39, wherein the peptide is comprised by a tetrameric complex.

ABSTRACT OF THE DISCLOSURE

This invention uses our knowledge of the mechanisms by which antigen is recognized by T cells to develop epitope-based vaccines directed towards HBV. More specifically, this application communicates our discovery of pharmaceutical compositions and methods of use in the prevention and treatment of HBV infection.

Figure 1. Monte Carlo population coverage analysis for HBV candidate epitopes



Monte Carlo population coverage analysis for HBV candidate epitopes

Plot of total frequency of genotypes as a function of the number of HBV candidate epitopes bound by HLA-A and B alleles, in an average population. Genotype values were derived by averaging the gene frequencies in Caucasian, North American Black, Japanese, Chinese, and Hispanic populations. Also shown is the cumulative frequency of genotypes.

Using currently available HLA typing data, a residual fraction (about 15%) of the genes, in an average population, are unspecified. To arrive at 100% accounting of genes, a fraction of the residual has been added for each hit population cluster in proportion to the relative frequency of the cluster within the HLA specified population.

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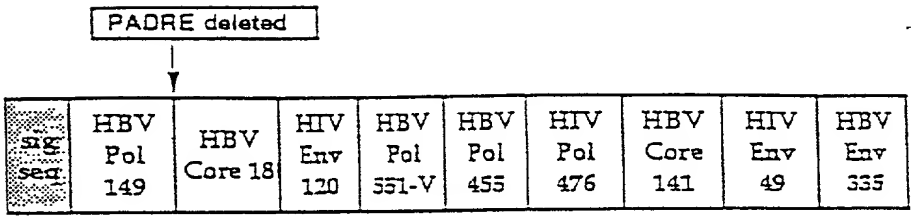
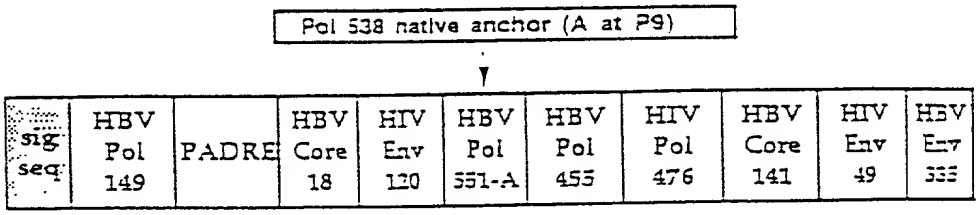
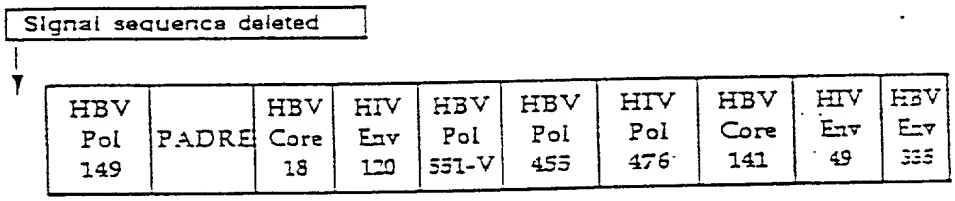


B. pMin.1-Anchor



C. pMin.1-No Sig



D. pMin.1-Switch

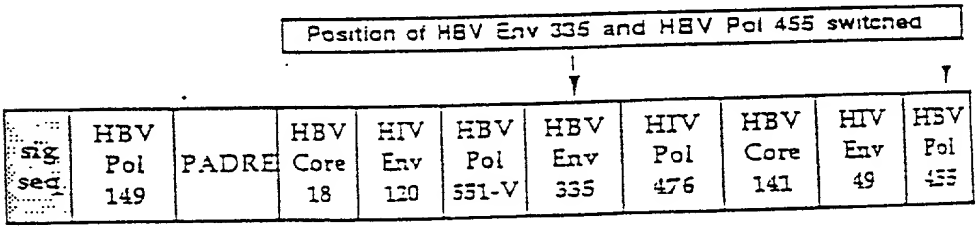


FIGURE 2

DECLARATION

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **INDUCING CELLULAR IMMUNE RESPONSES TO HEPATITIS B VIRUS USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS** the specification of which ____ is attached hereto or _X___ was filed on July 8, 1999 as Application No. _____ and was amended on _____ (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date
60/013,363	March 13, 1996

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status
08/820,360	March 12, 1997	Pending
09/189,702	November 10, 1998	
08/205,713	March 4, 1994	
08/159,184	November 29, 1993	
08/073,205	June 4, 1993	
08/027,146	March 5, 1993	

Full Name of Inventor 1:	Last Name: **SETTE**	First Name: **ALESSANDRO**	Middle Name or Initial:	
Residence & Citizenship:	City: **La Jolla**	State/Foreign Country: **California**	Country of Citizenship: **Italy**	
Post Office Address:	Post Office Address: **5551 Linda Rosa Avenue**	City: **La Jolla**	State/Country: **California**	Postal Code: **92037**
Full Name of Inventor 2:	Last Name: **SIDNEY**	First Name: **JOHN**	Middle Name or Initial:	
Residence & Citizenship:	City: **La Jolla**	State/Foreign Country: **California**	Country of Citizenship: **United States**	
Post Office Address:	Post Office Address: **4218 Corte de la Siena**	City: **La Jolla**	State/Country: **California**	Postal Code: **92130**
Full Name of Inventor 3:	Last Name: **SOUTHWOOD**	First Name: **SCOTT**	Middle Name or Initial:	
Residence & Citizenship:	City: **Santee**	State/Foreign Country: **California**	Country of Citizenship: **United States**	
Post Office Address:	Post Office Address: **10679 Strathmore Dr.**	City: **Santee**	State/Country: **California**	Postal Code: **92071**
Full Name of Inventor 4:	Last Name: **VITIELLO**	First Name: **MARIA**	Middle Name or Initial: **A.**	
Residence & Citizenship:	City: **La Jolla**	State/Foreign Country: **California**	Country of Citizenship: **Italy**	
Post Office Address:	Post Office Address: **7522 High Avenue**	City: **La Jolla**	State/Country: **California**	Postal Code: **92037**
Full Name of Inventor 5:	Last Name: **LIVINGSTON**	First Name: **BRIAN**	Middle Name or Initial: **D.**	
Residence & Citizenship:	City: **San Diego**	State/Foreign Country: **California**	Country of Citizenship: **United States**	
Post Office Address:	Post Office Address: **13555 Chaco Court**	City: **San Diego**	State/Country: **California**	Postal Code: **92129**
Full Name of Inventor 6:	Last Name: **CELIS**	First Name: **ESTEBAN**	Middle Name or Initial:	
Residence & Citizenship:	City: **Rochester**	State/Foreign Country: **Minnesota**	Country of Citizenship: **United States**	
Post Office Address:	Post Office Address: **3683 Wright Road S.W.**	City: **Rochester**	State/Country: **Minnesota**	Postal Code: **55902**
Full Name of Inventor 7:	Last Name: **KUBO**	First Name: **RALPH**	Middle Name or Initial: **T.**	
Residence & Citizenship:	City: **Carlsbad**	State/Foreign Country: **California**	Country of Citizenship: **United States**	
Post Office Address:	Post Office Address: **6921 Pear Tree Drive**	City: **Carlsbad**	State/Country: **California**	Postal Code: **92009**
Full Name of Inventor 8:	Last Name: **GREY**	First Name: **HOWARD**	Middle Name or Initial: **M.**	
Residence & Citizenship:	City: **La Jolla**	State/Foreign Country: **California**	Country of Citizenship: **United States**	
Post Office Address:	Post Office Address: **1461 Caminito Batea**	City: **La Jolla**	State/Country: **California**	Postal Code: **92037**
Full Name of Inventor 9:	Last Name: **CHESNUT**	First Name: **ROBERT**	Middle Name or Initial: **W.**	
Residence & Citizenship:	City: **Cardiff-by-the-Sea**	State/Foreign Country: **California**	Country of Citizenship: **United States**	
Post Office Address:	Post Office Address: **1473 Kings Cross Drive**	City: **Cardiff-by-the-Sea**	State/Country: **California**	Postal Code: **92007**

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1 ALESSANDRO SETTE	Signature of Inventor 2 JOHN SIDNEY	Signature of Inventor 3 SCOTT SOUTHWOOD
Date	Date	Date
Signature of Inventor 4 MARIA A. VITIELLO	Signature of Inventor 5 BRIAN D. LIVINGSTON	Signature of Inventor 6 ESTEBAN CELIS
Date	Date	Date
Signature of Inventor 7 RALPH T. KUBO	Signature of Inventor 8 HOWARD M. GREY	Signature of Inventor 9 ROBERT W. CHESNUT
Date	Date	Date

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